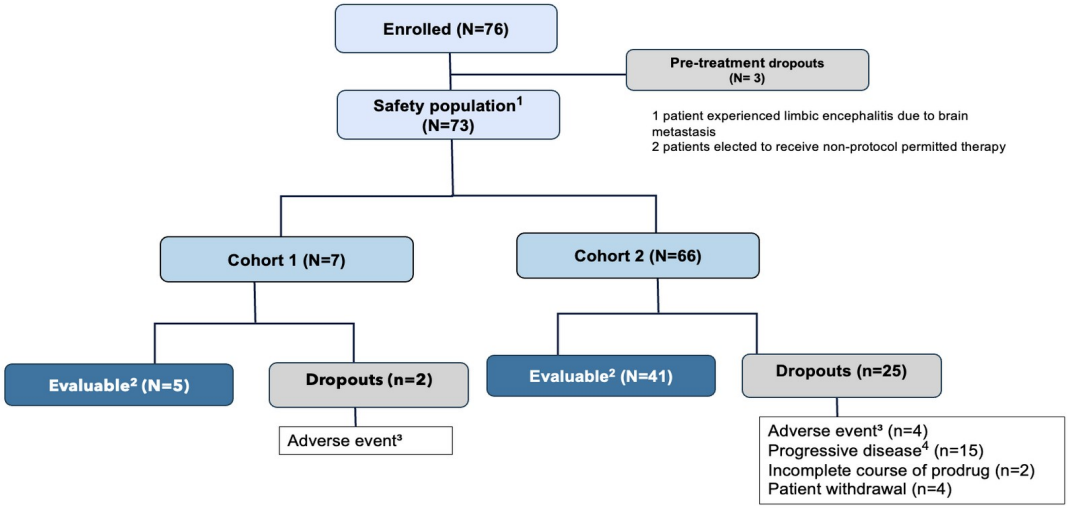
Supplemental Figure 1

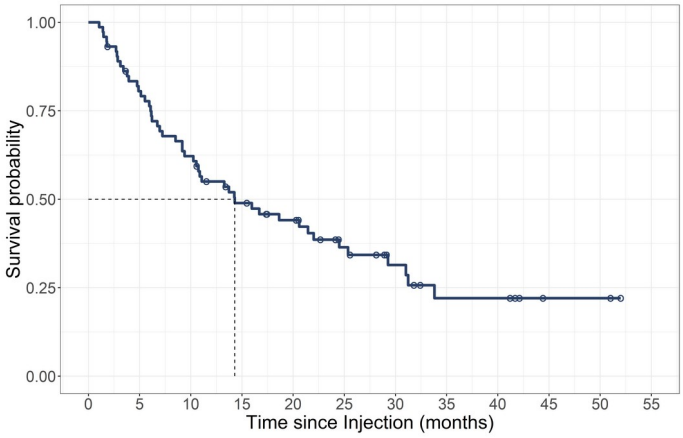
a.



[1]Safety population: Received at least 1 injection of CAN-2409
[2]Evaluable patients are those who received 2 courses of Aglatimagene + prodrug (valacyclovir) and completed the 12-week treatment window
[3]Adverse event: Cohort 1 (Pneumonitis G3 possibly related to study drug , Pulmonary embolism G3 unrelated to study drug), Cohort 2 (empyema grade 3 possibly related to study drug, pneumonia grade 3, pre-syncope grade 2 and bullous dermatitis grade 3 unrelated to study drug)
[4] Physician choice of change of therapy due to PD or suspicion of PD, however pseudo progression may have contributed

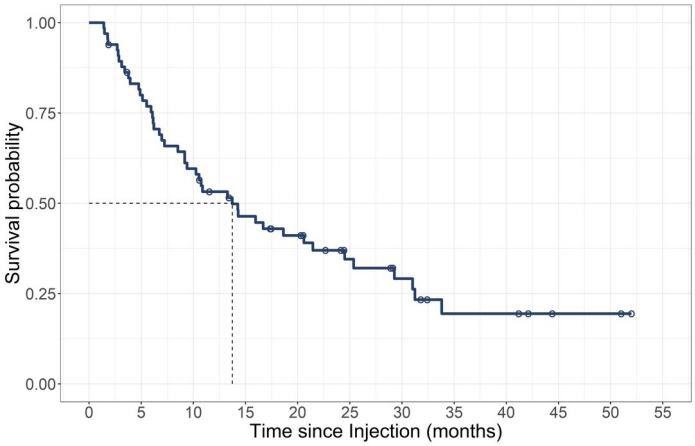
b.

Overall Survival of safety population for cohort 1 and 2



c.

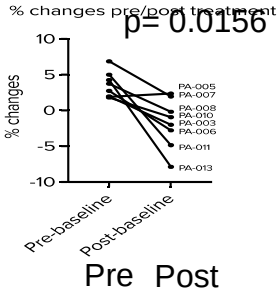
Overall Survival of safety population for cohort 2



d.

Population	PDL1 Subgroup	Number of Patients	Median OS (month)
Cohort II Evaluable	< 1%	16	19.4
Cohort II Evaluable	1 - 49%	13	20.6
Cohort II Evaluable	>= 50%	8	21.45

e.



Supplemental Figure 1 | CONSORT diagram and survival of safety population.
a, Consort diagram for all patients enrolled and included in the safety population (received at least 1 aglatimagene besadenovec injection). **b,** Kaplan-Meier curve of the overall survival for the safety population in cohort 1 and 2 (N=73). **c,** Kaplan-Meier curve of the overall survival for the safety

population in Cohort 2 (*n*=66). **d,** Table of median overall survival (months) on PDL1 status of <1 percent, 1-59 percent and ≥50 percent for all cohort 2 evaluable patients. **e,** Graph illustrating percent changes in tumor growth rate calculated as percentage increase of the sum of diameters over time. Comparison between pre- and post-injection changes for patients with pre-treatment scans available for analysis (*p*=0.0156).

Supplemental Table 1: List of research sites and site principal investigators with enrolled patients

Principal Investigator	Site
Leigh Swartz, MD	Hunter Holmes McGuire VA Medical Center
Wallace Akerley, MD	Huntsman Cancer Institute, University of Utah
Janani Reisenauer, MD	Mayo Clinic, Rochester MN
Daniel Sterman, MD	New York University Langone Health
Christine Bestvina, MD	The University of Chicago
Charu Aggarwal, MD	The University of Pennsylvania
George Eapen, MD	The University of Texas MD Anderson Cancer Center
Omar Ibrahim, MD	UConn Health
Ranee Mehra, MD	University of Maryland, Baltimore
Fabien Maldonado, MD	Vanderbilt University Medical Center
Erin Alesi, MD	Virginia Commonwealth University

Supplemental Table 1 | List of research sites and site principal investigators with enrolled patients. Table listing Principal Investigators at each research site and their location.

Supplemental Table 2
Overall Demographics and clinical characteristics

Baseline demographics and clinical characteristics	Enrolled n=76 (%)	Safety n=73 (%)	Evaluable n= 46 (%)
Age			
Median (Range)	67 (43-88)	67 years (43-88)	69 (43-84)
Sex			
Female	34 (44.7%)	32 (44%)	22 (47.8%)
Male	42 (55.3%)	41 (56%)	24 (52.2%)
Race			
Black/African American	10 (13.2%)	9 (12%)	7 (15.2%)
Asian	1 (1.3%)	1 (1%)	1 (2.2%)
White	61 (80.3%)	59 (81%)	37 (80.4%)
Unknown	4 (5.3%)	4 (5%)	1 (2.2%)
Ethnicity			
Not Hispanic or Latino	67 (88.2%)	66 (90%)	41 (89.1%)
Not reported	9 (11.8%)	7 (10%)	5 (10.9%)
ECOG			
0	33 (43.4%)	32(44%)	24 (52.2%)
1	43 (56.6%)	41(56%)	22 (47.8%)
Histology			
Squamous	16 (21.3%)	16 (22%)	9 (19.6%)
Non-Squamous	59 (78.7%)	57(78%)	37 (80.4%)
PD-L1 Expression			
<1%	35 (46.0%)	35 (48%)	21 (45.7%)
1 – 49 %	17 (22.4%)	15 (21%)	13 (28.3%)
≥ 50 %	20 (26.3%)	19 (26%)	8 (17.4%)
Unknown	4 (5.3%)	4 (5%)	4 (8.7%)
Stage			
Stage III	7 (9.2%)	7 (10%)	6 (13.0%)
Stage IV	69 (90.8%)	66 (90%)	40 (87.0%)
Smoking History			
Never	7 (9.2%)	6 (8%)	4 (8.7%)
Former	60 (78.9%)	58 (79%)	38 (82.6%)
Current	9 (11.8%)	9 (12%)	4 (8.7%)
Treatment Regimen at Enrollment			
Single ICI	53 (69.7%)	50 (68%)	30 (65.2%)
ICI + chemotherapy	23 (30.3%)	23 (32%)	16 (34.8%)
ICI Regimen			
Durvalumab	3 (3.9%)	3 (4%)	3 (6.5%)
Nivolumab	5 (6.6%)	5 (7%)	3 (6.5%)
Pembrolizumab	68 (89.5%)	65 (89%)	40 (87.0%)
Prior Lines of Treatment			
None	10 (13.2%)	10 (14%)	6 (13.0%)
Platinum-based with pemetrexed	44 (57.9%)	44 (60%)	26 (56.5%)
Platinum-based with taxane	15 (19.7%)	15 (21%)	11 (23.9%)
Platinum-based and/or other	4 (5.3%)	4 (5%)	3 (6.5%)
Unknown	3 (3.9 %)	0 (0%)	0 (0%)

Supplemental Table 2 | Overall baseline demographics and clinical characteristics for enrolled population (n=76), safety population (n=73), and evaluable population (n=46). Key demographic information is listed with patient numbers and percentages of total patients for each category.

Supplemental Table 3: Treatment-related adverse events (occurring in ≥5% patients)

Most common treatment-emergent related adverse events occurring in ≥ 5% patients, n=73				
SOC/Adverse Event Term (v5.0)	**Grade 1 n (%)**	**Grade 2 n (%)**	**Grade 3 n (%)**	**Total n (%)**
Gastrointestinal disorders				
Diarrhea	5 (7)	0 (0)	0 (0)	5 (7)
Nausea	11 (15)	4 (5)	0 (0)	15 (21)
Vomiting	4 (5)	2 (3)	0 (0)	6 (8)
General disorders and administration site conditions				
Chills	8 (11)	0 (0)	0 (0)	8 (11)
Fatigue	16 (22)	7 (10)	0 (0)	23 (32)
Influenza like illness	3 (4)	1 (1)	0 (0)	4 (5)
Pyrexia	12 (16)	1 (1)	1 (1)	14 (19)
Investigations				
Aspartate aminotransferase increased	4 (5)	0 (0)	0 (0)	4 (5)
Blood creatinine increased	4 (5)	3 (4)	0 (0)	7 (10)
Metabolism and nutrition disorders				
Decreased appetite	2 (3)	4 (5)	0 (0)	6 (8)
Nervous system disorders				
Headache	3 (4)	1 (1)	0 (0)	4 (5)
Respiratory, thoracic and mediastinal disorders				
Dyspnoea	2 (3)	4 (5)	0 (0)	6 (8)
Pneumonitis	0 (0)	2 (3)	2 (3)	4 (5)

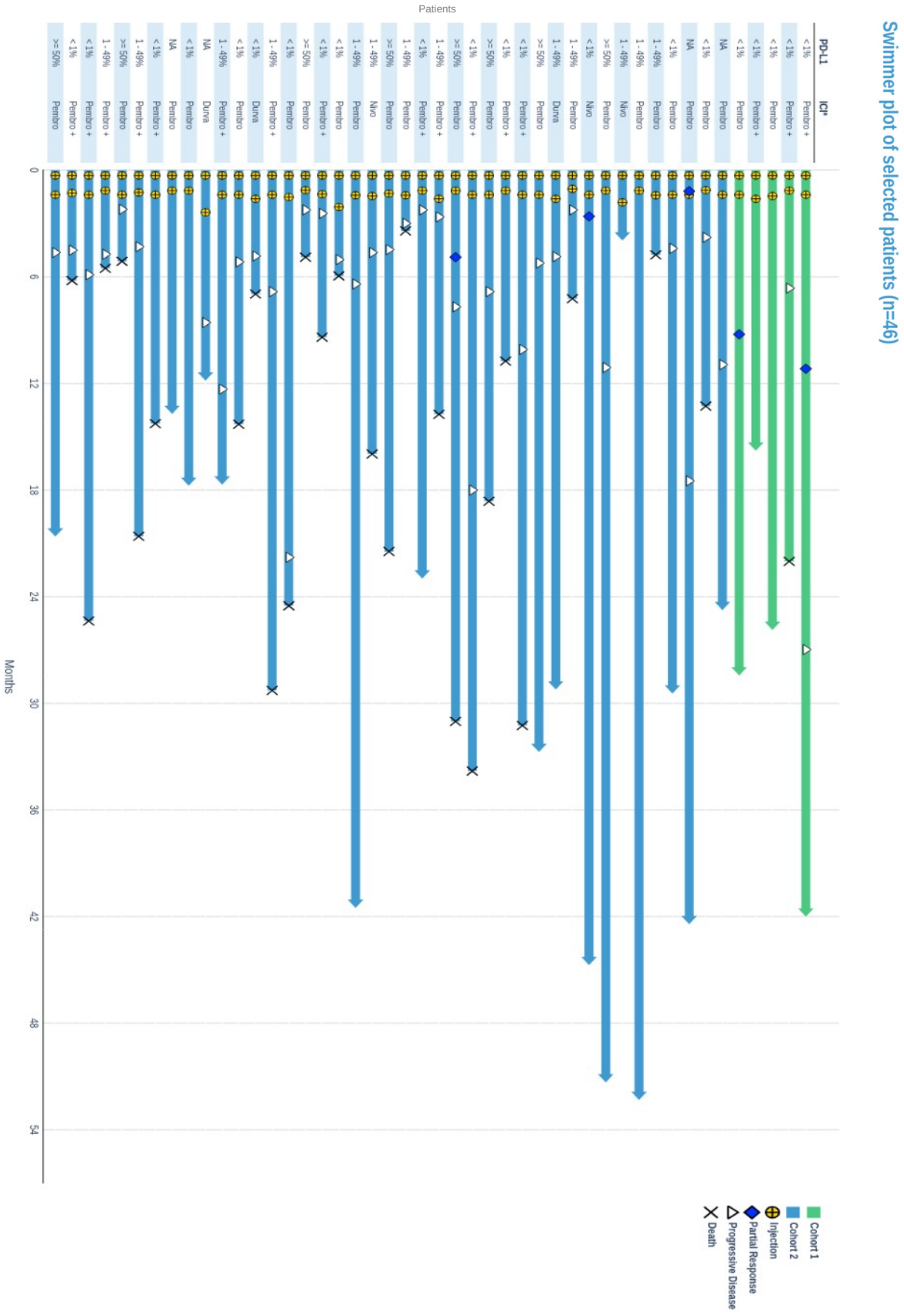
Supplemental Table 3 | Treatment-related adverse events (occurring in ≥5% patients). Adverse events categorized by SOC (System Order Class)/Adverse Event Term and grade, with total number of patients and percent of total listed.

Supplemental Table 4: Treatment emergent, related adverse events grade ≥3

Treatment-emergent related adverse events ≥ grade 3, n=73		
SOC/Adverse Event Term (v5.0)	**Grade 3 n (%)**	**Total n (%)**
General disorders and administration site conditions		
Inflammation	1 (1)	1 (1)
Pyrexia	1 (1)	1 (1)
Infections and infestations		
Empyema	1 (1)	1 (1)
Investigations		
Blood alkaline phosphatase increased	1 (1)	1 (1)
Lymphocyte count decreased	1 (1)	1 (1)
Platelet count decreased	1 (1)	1 (1)
Respiratory, thoracic and mediastinal disorders		
Bronchial hyperreactivity	1 (1)	1 (1)
Hypoxia	1 (1)	1 (1)
Pneumonitis	2 (3)	2 (3)

Supplemental Table 4 | Treatment-related adverse events grade ≥3. Treatment-emergent events that are grade 3 and higher are categorized by SOC/Adverse Event Term and grade, with total number of patients and percent of total listed.

Supplemental Figure 2



Supplemental Figure 2 | Swimmer Plot of evaluable patients in Cohorts 1 and 2 (n=46). Swimmer plot with Subject ID, PD-L1 expression (categorized into <1%, 1-49%, and ≥50%), and ICI

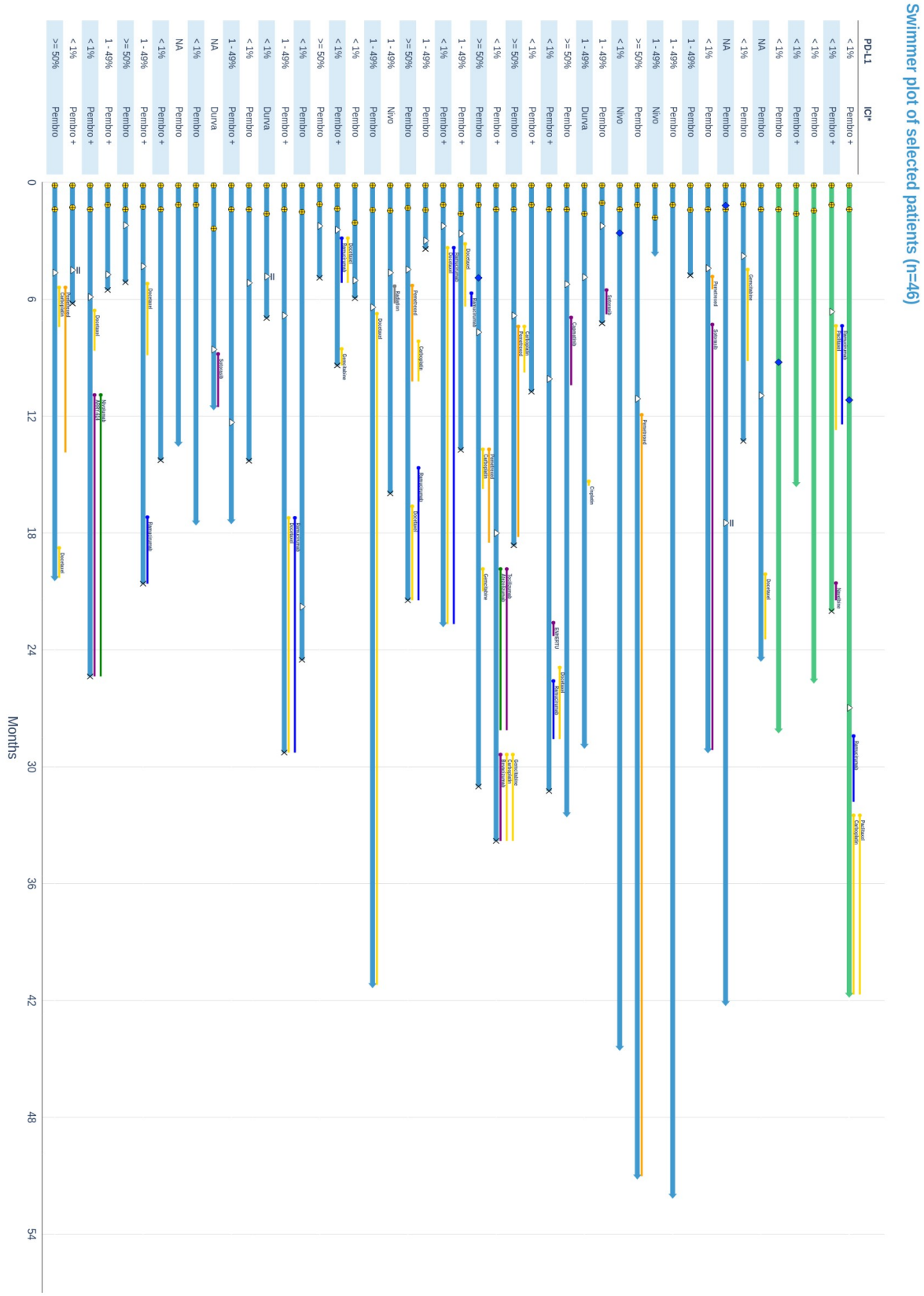
(immune checkpoint inhibitor therapy) each patient was taking at the time of enrollment. Pembro is pembrolizumab, Durva indicates durvalumab, and Nivo is for Nivolumab. A "+" represents that the patient was also on chemotherapy at enrollment. Lines in green represent overall survival for Cohort 1 patients with Cohort 2 patients in blue, where right facing arrows represent that they are still alive at the time of data cutoff. Death events are marked with an "X", progressive disease by a white triangle, partial response by a blue diamond, and injection times are represented by yellow circles. The 46 evaluable patients from Cohorts 1 and 2 are represented on the plot.

Supplemental Table 5: Post-progression treatment

Post progression additional therapy	Evaluable with progression (n,%)	Mid-treatment dropout (n,%)
Chemotherapy	6 (17.1)	3 (13.0)
Chemotherapy + IO	4 (11.4)	1 (4.3)
Chemotherapy + other	1 (2.9)	0 (0)
Chemotherapy + Ramucirumab	9 (25.7)	4 (17.4)
Discontinued all treatment	7 (20.0)	7 (30.4)
No changes	3 (8.6)	3 (13.0)
Other	2 (5.7)	1 (4.3)
Radiation	1 (2.9)	0 (0)
Radiation and other	1 (2.9)	0 (0)
Unknown	1 (2.9)	4 (17.4)
Total	**35 (100)**	**23 (100)**
Total	**35 (100)**	**23 (100)**

Supplemental Table 5 | Post-progression treatment of evaluable patients (cohort 1 and 2) with progressive disease (*n*=35). Therapies started after progression are listed for patients experiencing progression for both evaluable and safety populations in cohorts 1 and 2. The number of patients for each therapy type and the percentage of total are listed.

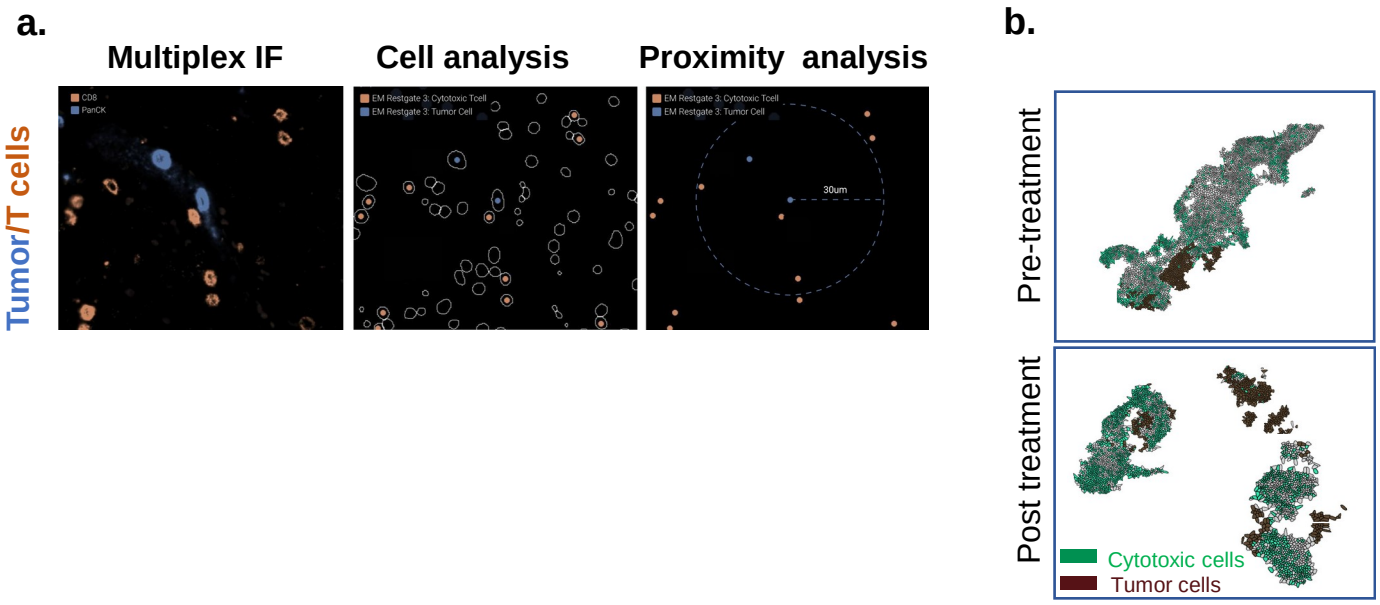
Supplemental Figure 3



Supplemental Figure 3 | Swimmers Plot with display of post-progression treatment of evaluable patients in Cohorts 1 and 2 (*n*=46).

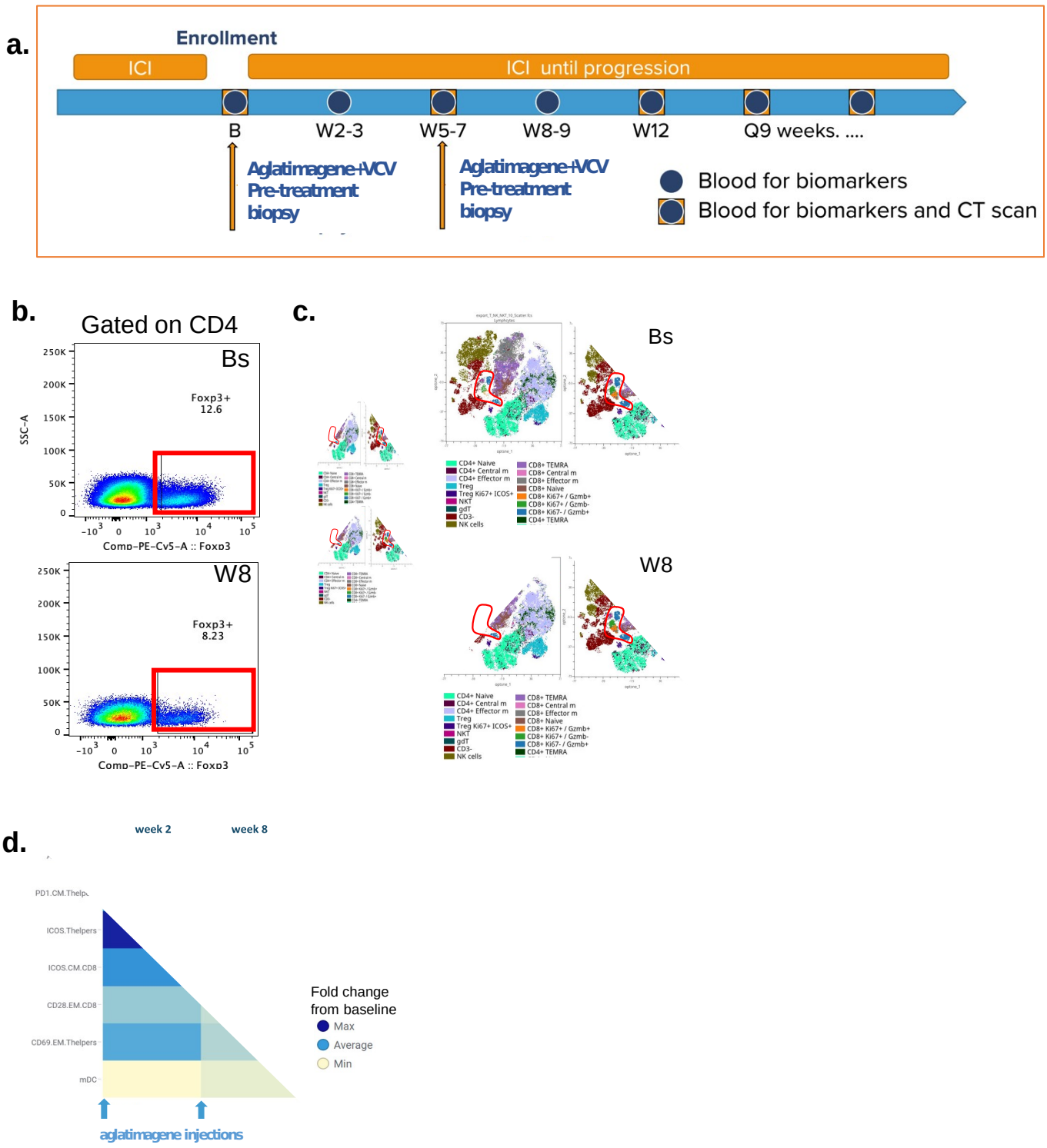
Swimmer plot with Subject ID, PD-L1 expression (categorized into <1%, 1-49%, and ≥50%), and ICI (immune checkpoint inhibitor therapy) each patient was taking at the time of enrollment. Pembro is pembrolizumab, Durva indicates durvalumab, and Nivo is for Nivolumab. A "+" represents that the patient was also on chemotherapy at enrollment. Lines in green represent overall survival for Cohort 1 patients with Cohort 2 patients in blue, where right facing arrows represent that they are still alive at the time of data cutoff. Death events are marked with an "X", progressive disease by a white triangle, partial response by a blue diamond, and injection times are represented by yellow circles. The 46 evaluable patients from Cohorts 1 and 2 are represented on the plot. Post-progression treatments are represented by yellow for chemotherapy, orange for pemetrexed, blue for ramucirumab, green for a second line immunotherapy, gray for radiotherapy, purple for other types of therapy, and two black lines for no changes to pre-progression therapy.

Supplemental Figure 4



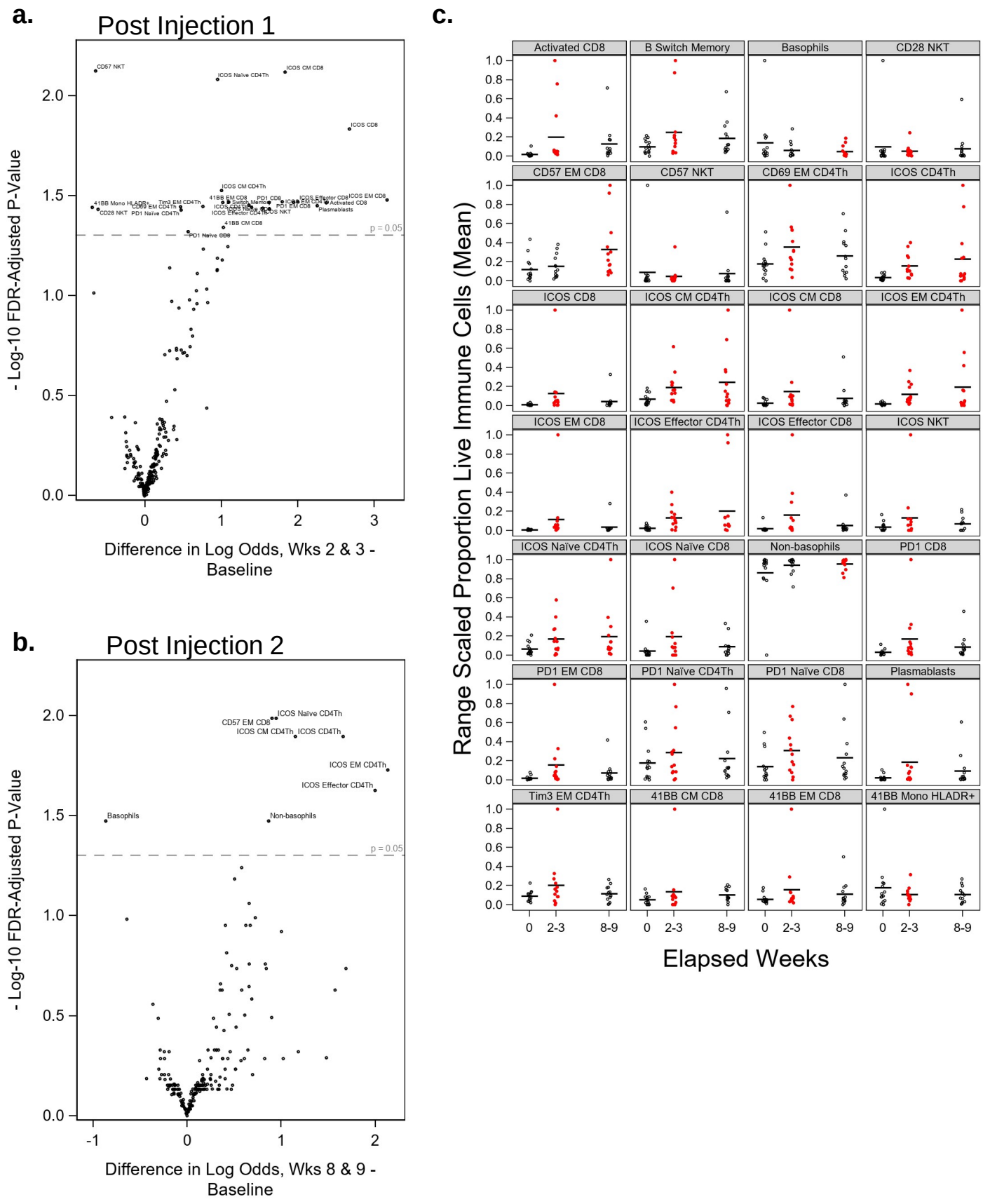
Supplemental Figure 4 | Multiplex Immunofluorescence identifies T cell proximities to tumor cells. a, Proximity analysis was applied to immunofluorescence (IF) stained samples to evaluate number of CD8+ cytotoxic cells within a distance of 100um radius from tumor cells. **b,** Voronoi diagram illustrating frequency analysis methodology in pre and post treatment samples. Areas identified as tumor cells are marked in brown while cytotoxic cells are marked in green.

Supplemental Figure 5



Supplemental Figure 5 | Blood and tissue sample collections and results. a, Treatment and sample collection schematic. **b,** Flow cytometry gating reveals a decrease in CD4+ FoxP3+ T regulatory cells after the second injection. **c,** t-SNE plot representing the T cell panel, highlighting increases observed in CD8+ GZMB+ Ki67+ T cells **d,** CyTOF shows similar increases in key T cell subtypes to flow cytometry results post 1st and 2nd injection. Fold change values from baseline represented by blue to yellow color scale.

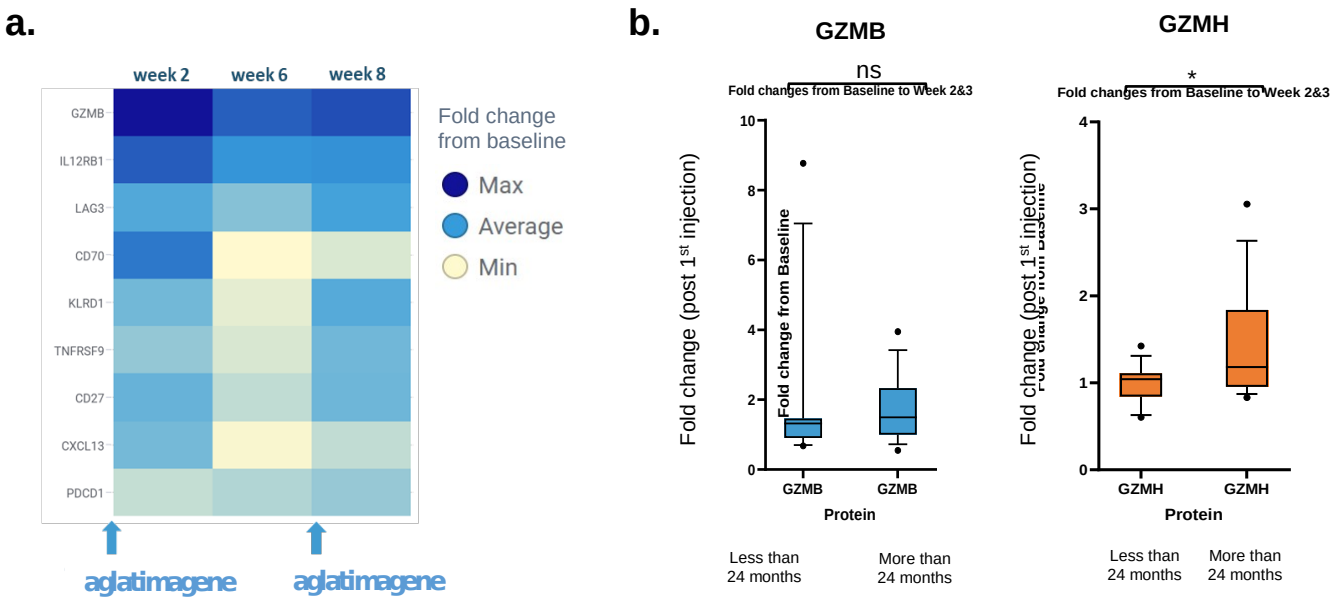
Supplemental Figure 6



Supplemental Figure 6 | CyTOF longitudinal changes. a, Volcano plot representing results of generalized linear mixed model applied to CyTOF frequency where each point corresponds to

differences in frequencies between baseline and post 1st injection for each measured cell phenotype. Points above the horizontal dotted line represent statistically significant differences (p <0.05) and cell types with a positive difference (on the right side of the plot) have higher expression after aglatimagene besadenovec injections. All treated patients represented, and FDR (False Discovery Rate) correction was applied in the calculation of p-values. **b,** Volcano plot as above for post 2nd injection differences from baseline. **c,** Range scaled proportion of live immune cells for each sample and timepoint. On the x axis 0 indicates baseline, 2-3 is post 1st injection, and 8-9 is post 2nd injection. The horizontal line marks the mean, and timepoints in red indicate significant differences from baseline per the GLMM results in the previous panel.

Supplemental Figure 7



Supplemental Figure 7 | Circulating protein changes. a, Fold changes from baseline of select immune markers measured using Olink® technology. Fold change values from baseline represented by blue to yellow color scale. **b,** Post 1st injection fold changes in GZMB were not significantly different between long and short surviving patients (<24 months $n = 16$, ≥24 months $n = 16$). Post 1st injection fold changes in GZMH were significantly higher in patients surviving ≥24 months than patients who died earlier than 24 months after start of treatment (asterisk indicates < 0.05, unpaired t-test p=0.0143). Box plots display the median for all evaluable patients who died before 24 months or survived longer than 24 months (GZMB $n = 16$, GZMH $n = 16$) with whiskers extending from the 10th to the 90th percentile. Data points outside this range are shown as individual dots representing outliers. GZMB data are represented in blue and GZMH data are in orange.

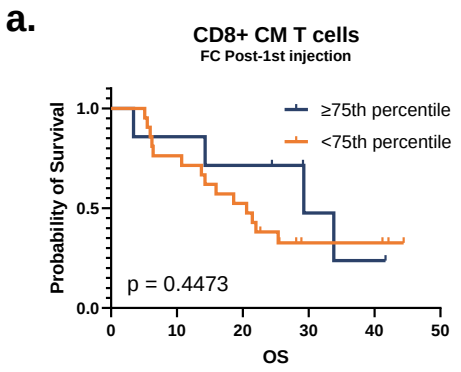
Supplemental Table 6

Biomarker and Timepoint	Directionality in Long Survivors	Total patients	Log-rank p-value
CyTOF – Baseline to post 1st injection			
CD69.TEMRA.Thelpers	**Higher**	23	0.0171
CD137.CM.CD8	Lower	12	0.0182
ICOS.Naive.Thelpers	**Higher**	21	0.0216
ICOS.Thelpers	**Higher**	23	0.0245
Ox40.CM.CD8	Lower	17	0.0316
CD57.CD8	**Higher**	23	0.0477
CyTOF - Baseline to post 2nd injection			
B.Memory.Switched	**Higher**	23	0.0325
CD11c.CM.Thelpers	Lower	22	0.0377
CD69.NK	**Higher**	23	0.0319
ICOS.TEMRA.Thelpers	**Higher**	21	0.0158
ICOS.Thelpers	**Higher**	23	0.0163
Ox40.CM.CD8	Lower	15	0.0005
PD1.CM.CD8	**Higher**	12	0.0315
PD1.EM.Thelpers	**Higher**	19	0.0303
PD1.TEMRA.Thelpers	**Higher**	16	0.0007
Tim3.CM.Thelpers	Lower	15	0.0494
Tim3.Naive.Thelpers	Lower	17	0.0127
Flow Cytometry – Baseline to post 1st injection			
CD56-CD16+NK Gzmb+Ki67+	Lower	28	0.0162
Flow Cytometry – Pre to post 2nd injection			
CD141+ DC	**Higher**	18	0.0044
CD1c+ DC CD14+ PDL1+	**Higher**	18	0.0473
Intermediate monocytes	Lower	18	0.0028
non-classical monocytes	Lower	18	0.0138
CD4 T	**Higher**	27	0.0293
CD4 T Central memory	**Higher**	27	0.0150
CD4 T Effector memory	**Higher**	27	0.0064
CD56-CD16+NK	**Higher**	27	0.0499
CD56+CD16-NK Gzmb+Ki67+	**Higher**	27	0.0159
CD8 T Naive	**Higher**	27	0.0363

Biomarker and Timepoint	Directionality in Long Survivors	Total patients	Log-rank p-value
Olink – Baseline to post 1st injection			
ANGPT2	Lower	35	0.0004
CCL23	Lower	35	0.0017
LAP.TGF.beta.1	Lower	35	0.0035
TNF	Lower	35	0.0046
PGF	Lower	35	0.0050
CSF.1	Lower	35	0.0074
CXCL13	Lower	35	0.0087
IL15	Lower	35	0.0104
ICOSLG	Lower	35	0.0140
TIE2	Lower	35	0.0208
MCP.1	Lower	35	0.0214
HGF	Lower	35	0.0248
PD.L2	Lower	35	0.0252
CD244	Lower	35	0.0306
CCL4	Lower	35	0.0321
TNFSF14	Lower	35	0.0357
CD4	Lower	35	0.0390
CD83	Lower	35	0.0407
MMP12	Lower	35	0.0427
Olink – Pre to post 2nd injection			
CD83	**Higher**	22	0.0342
CX3CL1	Lower	22	0.0478
GZMA	**Higher**	22	0.0476
IL12RB1	Lower	13	0.0230
MCP.3	**Higher**	22	0.0335

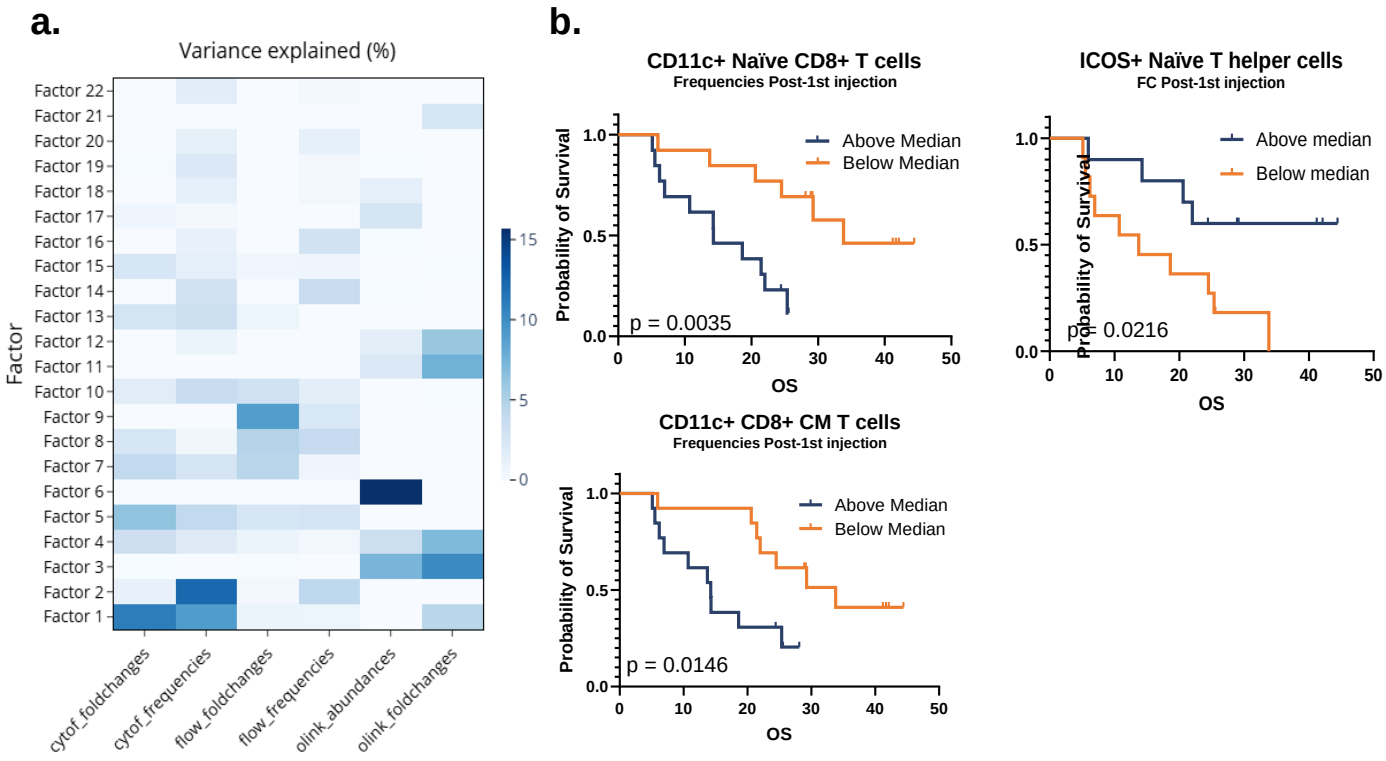
Supplemental Table 6 | Log-rank test results of post injection fold changes. Table describing significant results of log-rank tests of fold changes for CyTOF, Flow Cytometry, and Olink®. Biomarkers with data for <12 patients were excluded from analysis. Nominal p-values are presented.

Supplemental Figure 8



Supplemental Figure 8 | T cell changes after 2nd injection, but not first, are associated with survival. a, Kaplan-Meier plot demonstrating that fold changes of CD8+ CM T cells after the first injection were not significantly associated with survival when split by the top 25% and bottom 75% (median OS 29.3 vs. 20.6 months, *n* = 28, log-rank p = 0.4473).

Supplemental Figure 9

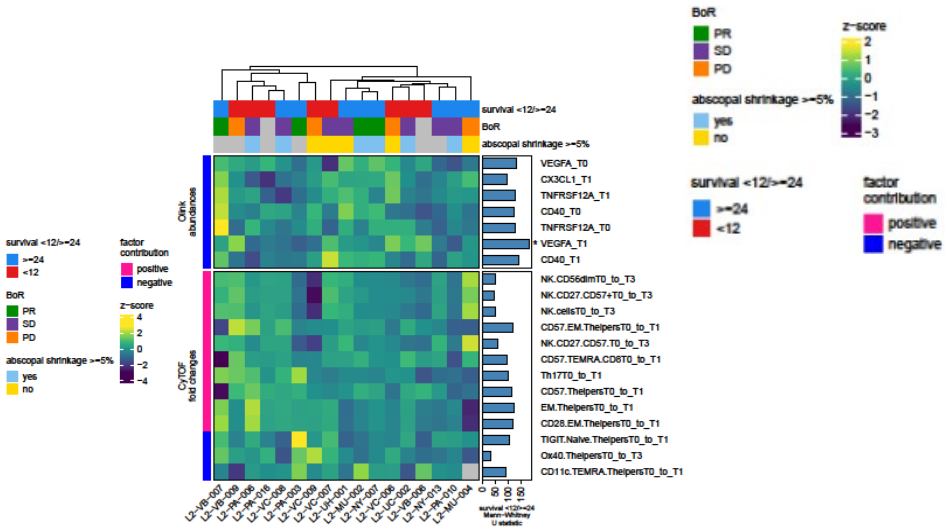


Supplemental Figure 9 | MOFA identifies cross-modality variation. A, Heatmap demonstrating the 22 distinct biological programs or factors that were identified by MOFA, with the contribution of each modality to each factor represented in darker shades of blue **b,** Kaplan-Meier plots representing additional cell types that were both identified in the top 10 top or bottom

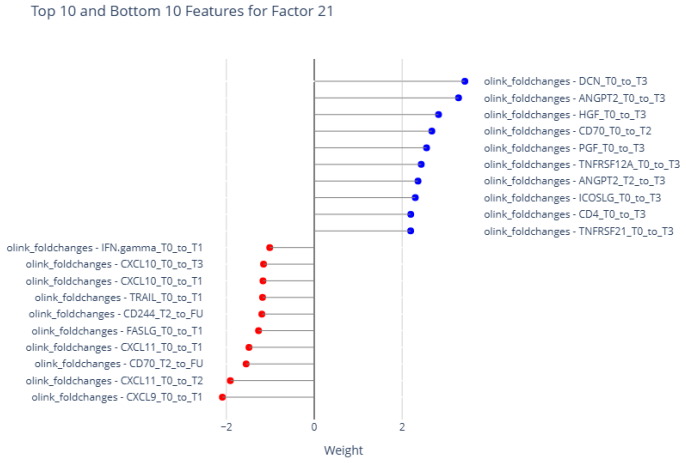
contributors to Factor 2 and are individually significantly associated with survival. Patients with lower post-1st injection frequencies in CD8+ CD11c+ Naïve T cells have significantly higher overall survival compared to patients with lower frequencies at the same timepoint (median OS 14.3 vs. 33.8 months, $n = 26$, log-rank p = 0.0035). Patients with higher post-1st injection fold changes in CD4+ ICOS+ Naïve T cells have significantly improved overall survival compared to patients with lower fold changes at the same timepoint (median OS not yet reached vs. 13.7 months, $n = 21$, log-rank p = 0.0216). Patients with lower post 1st injection frequencies in CD8+ CD11c+ CM T cells have significantly improved overall survival compared to patients with higher frequencies at the same timepoint (median OS 14.3 vs. 33.8 months, $n = 26$, log-rank p = 0.0146).

Supplemental Figure 10

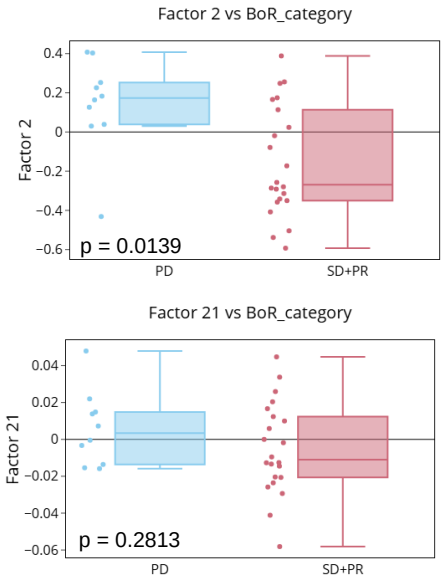
a.



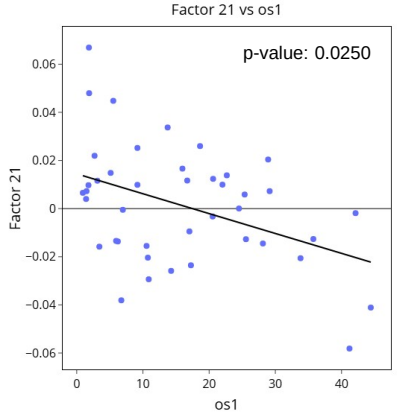
b.



c.



d.



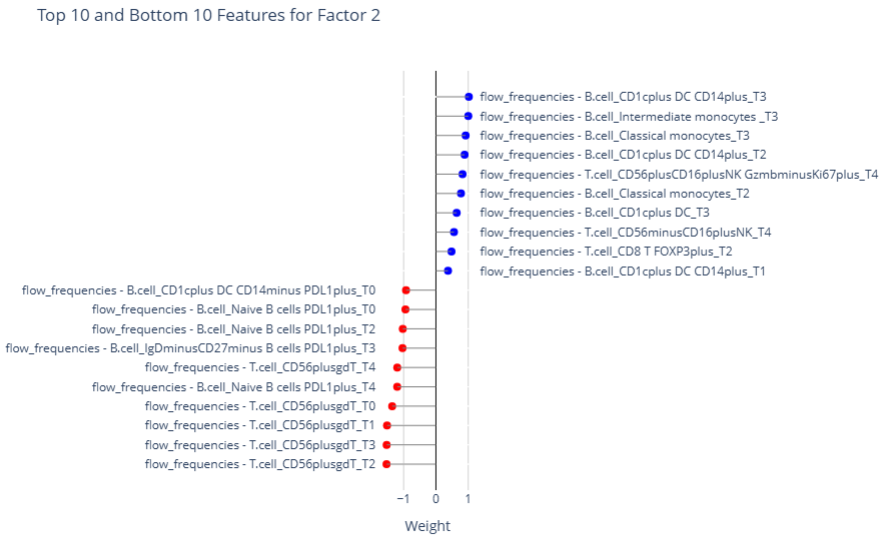
Supplemental Figure 10 | MOFA identifies Factor 21 and is associated with survival. a, Heatmap representing the 10 highest positive and negative contributing biomarkers to Factor 21, evaluable patients only, patients with >5 missing data points and patients with survival between 12-24 months were excluded. **b,** Lollipop diagram representing weights of the 10 highest positive and negative contributing biomarkers to Factor 21 from the Olink® fold changes modality. Blue dots to the right contribute positively to Factor 21 while red dots to the left contribute inversely. **c,** Factor 2 and Factor 21 values are lower in patients who have stable disease (SD) or partial response (PR) than in patients with progressive disease (PD) as their best overall response (BoR) Mann-Whitney U test $p = 0.0139$ and $p = 0.2813$, respectively). **d,** Scatter plot representing all patients with blue dots and regression line shows inverse correlation between Factor 21 and overall survival (Spearman r = -0.35, p = 0.0250).

Supplemental Figure 11

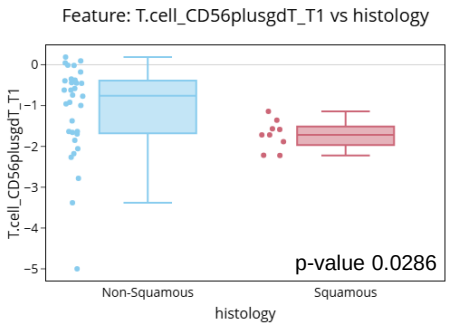
a.

Biomarker	Difference	p-value
Baseline differences by histology		
CD4+ T Naïve	2.187043	0.003895
CD4+ T Central memory	1.781342	0.004901
CD4+ T Ki67-IFNg+	2.321972	0.009463
Treg Naïve	2.085071	0.00949
non-classical monocytes	2.194788	0.010598
CD56+CD16+NK GZMB+Ki67+	1.724758	0.011235
CD4+ T	1.087532	0.018221
CD4+ T TEMRA	1.355774	0.023844
Treg Central memory	1.306984	0.02994
NKT	1.088438	0.040021
pDC	1.346783	0.04391
pDC PDL1+	2.340551	0.047716
CD56-gdT GZMB-Ki67+	-2.238124	0.013263
IgD-CD27- B cells Ki67+	-1.747835	0.025395
Post 2nd injection fold change differences by histology		
Non-switched memory B cells PDL1+	1.605067	0.010435
CD1c+ CD14+ DCs	1.253126	0.014345
Naïve CD8+ T cells	0.925061	0.038284
Non-switched memory B cells	1.563910	0.043025
CD4+ EM T cells	0.885191	0.044517

b.



c.



Supplemental Figure 11 | Flow frequencies from Factor 2 are associated with histology. **a,** Table listing differences and p-values from unpaired t-tests performed on baseline values and post-2nd injection fold changes of cell types identified through flow cytometry analysis. **b**, Lollipop diagram representing weights of the 10 highest positive and negative contributing biomarkers to

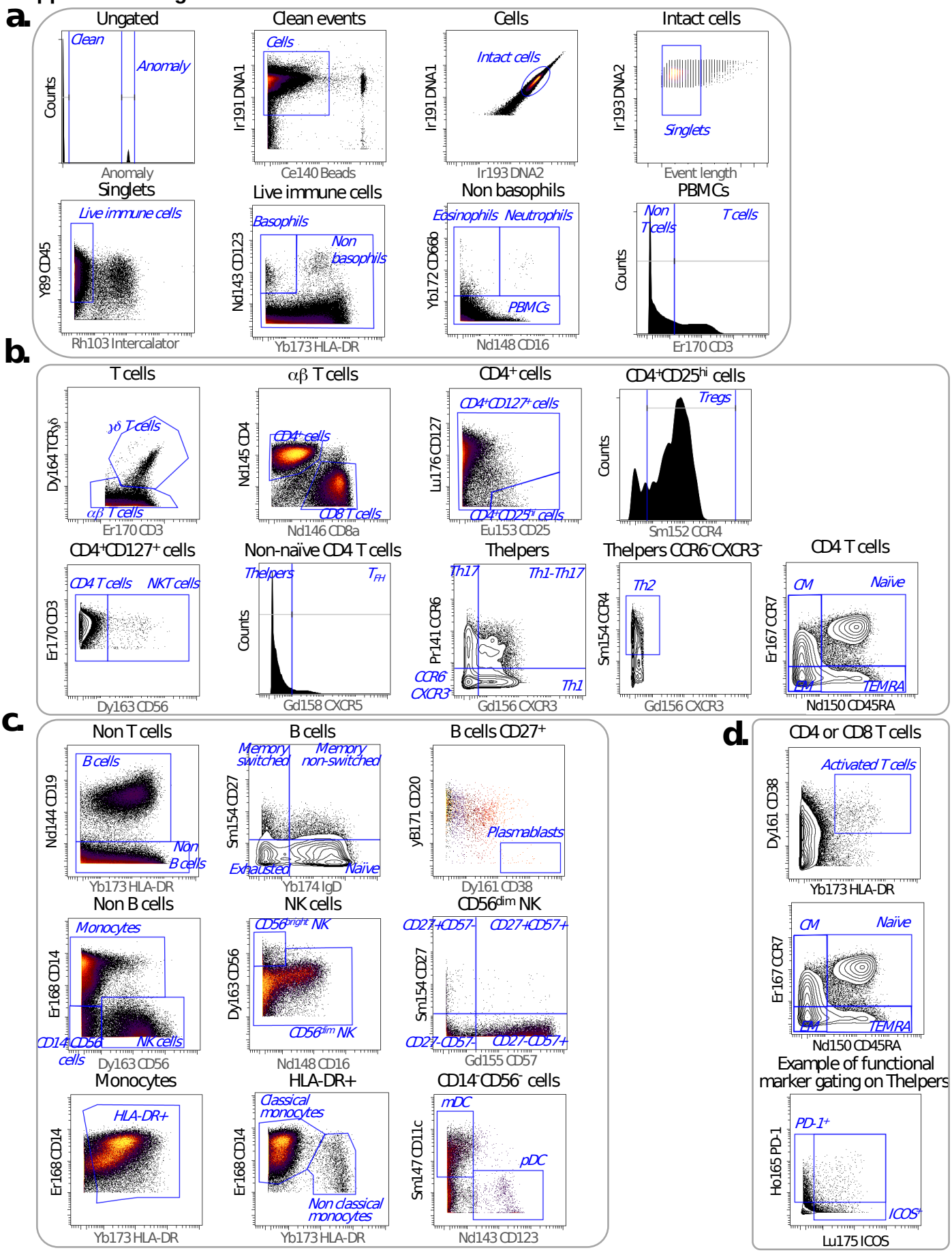
Factor 2 from the flow cytometry frequencies modality. Blue dots to the right contribute positively to Factor 21 while red dots to the left contribute inversely. **c,** CD56+ γδT cells are significantly higher after the first injection in non-squamous patients than squamous patients (Mann-Whitney U test, $p = 0.0286$).

Supplemental Figure 12

Mass	Target
Y89	CD45
Rh103	Live/Dead
Ce140	EQ Beads
Pr141	CD196 /CCR6
Nd142	CD134/OX40
Nd143	CD123
Nd144	CD19
Nd145	CD4
Nd146	CD8a
Sm147	CD11c
Nd148	CD16
Sm149	CD45RO
Nd150	CD45RA
Eu151	CD161
Sm152	CD194/CCR4
Eu153	CD25
Sm154	CD27
Gd155	CD57
Gd156	CD183/CXCR3
Gd157	PD-L1
Gd158	CD185/CXCR5
Tb159	TIGIT
Gd160	CD28
Dy161	CD38
Dy162	CD69
Dy163	CD56/NCAM
Dy164	TCRγδ
Ho165	CD279/PD-1
Er166	CD294/CRTH2
Er167	CD197/CCR7
Er168	CD14
Tm169	CD366/Tim-3
Er170	CD3
Yb171	CD20
Yb172	CD66b
Yb173	HLA-DR
Yb174	IgD
Lu175	CD278/ICOS
Yb176	CD127
Ir191	DNA1
Ir193	DNA2
Bi209	CD137/4-1BB

Supplemental Figure 12 | Table describing the antibodies used for CyTOF analysis. Mass and target are included for each cell marker assessed.

Supplemental Figure 13



Supplemental Figure 13 | CyTOF gating schema. a, Initial gating for PBMCs is shown in the first panel. **b,** Gating for T cells and **c,** non-T cells are shown in the following panels. **d,** Activation markers and functional marker gating examples on T cells are shown.