(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 3 934 673 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
07.01.2026 Bulletin 2026/02

(21) Application number: **20704372.0**

(22) Date of filing: **10.01.2020**

(51) International Patent Classification (IPC):
A61K 35/768 *(2015.01)* ***A61K 31/7105*** *(2006.01)*
C07K 14/54 *(2006.01)* ***C12N 15/86*** *(2006.01)*
C12N 15/88 *(2006.01)*

(52) Cooperative Patent Classification (CPC):
C12N 15/86; A61K 31/7105; A61K 35/768; C07K 14/5434; C12N 15/88; C12N 2770/36121; C12N 2770/36132

(86) International application number:
PCT/US2020/013069

(87) International publication number:
WO 2020/185293 (17.09.2020 Gazette 2020/38)

(54) SYNTHETIC ONCOLYTIC LNP-REPLICON RNA AND USES FOR CANCER IMMUNOTHERAPY

SYNTHETISCHE ONKOLYTISCHE LNP-REPLICON-RNA UND VERWENDUNGEN ZUR KREBSIMMUNTHERAPIE

ARN RÉPLICON LNP ONCOLYTIQUE SYNTHÉTIQUE ET UTILISATIONS EN IMMUNOTHÉRAPIE ANTICANCÉREUSE

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **08.03.2019 US 201962815611 P**

(43) Date of publication of application:
12.01.2022 Bulletin 2022/02

(73) Proprietors:
- **Massachusetts Institute of Technology**
  Cambridge, MA 02139 (US)
- **Ohio State Innovation Foundation**
  Columbus, OH 43201 (US)

(72) Inventors:
- **IRVINE, Darrell, J.**
  Arlington, MA 02476 (US)
- **WITTRUP, Karl, Dane**
  Chestnut Hill, MA 02467 (US)
- **WEISS, Ron**
  Newton, MA 02459 (US)
- **LI, Yingzhong**
  Quincy, MA 02171 (US)
- **MOMIN, Noor**
  Cambridge, MA 02139 (US)
- **DONG, Yizhou**
  Dublin, OH 43017 (US)

(74) Representative: **HGF**
HGF Limited
4th Floor, 1 City Square
Leeds LS1 2AL (GB)

(56) References cited:
WO-A1-2016/037053 WO-A2-2012/006376

- **NADIA LUHESHI ET AL: "Abstract 5017: MEDI1191, a novel IL-12 mRNA therapy for intratumoral injection to promote T H 1 transformation of the patient tumor microenvironment", IMMUNOLOGY, 1 July 2019 (2019-07-01), pages 5017 - 5017, XP055682617, DOI: 10.1158/1538-7445.AM2019-5017**

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Processed by Luminess, 75001 PARIS (FR)

(Cont. next page)

- **H. REN ET AL: "Immunogene therapy of recurrent glioblastoma multiforme with a liposomally encapsulated replication-incompetent Semliki forest virus vector carrying the human interleukin-12 gene - a phase I/II clinical protocol", JOURNAL OF NEURO-ONCOLOGY., vol. 64, no. 1-2, 1 August 2003 (2003-08-01), US, pages 147 - 154, XP055682947, ISSN: 0167-594X, DOI: 10.1007/BF02700029**
- **KENNETH LUNDSTROM: "Replicon RNA Viral Vectors as Vaccines", VACCINES, vol. 4, no. 4, 7 November 2016 (2016-11-07), pages 39, XP055552221, DOI: 10.3390/vaccines4040039**
- **IAN LAI ET AL: "Lipid nanoparticles that deliver IL-12 messenger RNA suppress tumorigenesis in MYC oncogene-driven hepatocellular carcinoma", JOURNAL FOR IMMUNOTHERAPY OF CANCER, BIOMED CENTRAL LTD, LONDON, UK, vol. 6, no. 1, 20 November 2018 (2018-11-20), pages 1 - 11, XP021262851, DOI: 10.1186/S40425-018-0431-X**
- **WEIYU ZHAO ET AL: "Lipid Polymer Hybrid Nanomaterials for mRNA Delivery", CELLULAR AND MOLECULAR BIOENGINEERING, vol. 11, no. 5, 19 June 2018 (2018-06-19), Boston, pages 397 - 406, XP055536695, ISSN: 1865-5025, DOI: 10.1007/s12195-018-0536-9**
- **ANONYMOUS: "Koch Institute Immune Engineering Symposium 2019", 6 February 2019 (2019-02-06), XP055682910, Retrieved from the Internet <URL:https://acir.org/weekly-digests/2019/february/koch-institute-immune-engineering-symposium-2019> [retrieved on 20200403]**
- **NOOR MOMIN ET AL: "Anchoring of intratumorally administered cytokines to collagen safely potentiates systemic cancer immunotherapy", SCIENCE TRANSLATIONAL MEDICINE, vol. 11, no. 498, 26 June 2019 (2019-06-26), US, pages eaaw2614, XP055655658, ISSN: 1946-6234, DOI: 10.1126/scitranslmed.aaw2614**
- **LUNDSTROM KENNETH: "Alphavirus-based vaccines", CURRENT OPINION IN MOLECULAR THERAPEUTICS, CURRENT DRUGS, LONDON, GB, vol. 4, no. 1, 1 February 2002 (2002-02-01), pages 28 - 34, XP008095711, ISSN: 1464-8431**
- **LUNDSTROM KENNETH ED - ORTEGO JAVIER ET AL: "Alphavirus vectors as tools in neuroscience and gene therapy", VIRUS RESEARCH, AMSTERDAM, NL, vol. 216, 22 August 2015 (2015-08-22), pages 16 - 25, XP029472298, ISSN: 0168-1702, DOI: 10.1016/J.VIRUSRES.2015.08.015**

Remarks:

The complete document including Reference Table(s) and the Sequence Listing(s) can be downloaded from the EPO website

Description

RELATED APPLICATION

[0001] This application claims priority under 35 U.S.C. § 119(e) to United States Provisional Application Serial Number 62/815,611, filed March 8, 2019.

BACKGROUND

[0002] The present disclosure relates to synthetic oncolytic viruses comprising a lipid nanoparticle comprising one or more types of lipid and a self-amplifying replicon RNA comprising a sequence that encodes an immunomodulatory molecule.

[0003] Lundstrom K et al, Vaccines (2016) vol 4, no. 4, page 39, provides a review of replicon RNA viral vectors as vaccines. Lai, I et al, Journal for ImmunoTherapy of Cancer (2018) vol 6, no. 1:125, page 1-11, reports that lipid nanoparticles that deliver IL-12 messenger RNA suppress tumorigenesis in MYC oncogene-driven hepatocellular carcinoma.

SUMMARY OF THE INVENTION

[0004] The invention is as set out in the claims.

[0005] Accordingly, the invention provides a synthetic oncolytic virus comprising:

(i) a lipid nanoparticle comprising one or more types of lipid, wherein the one or more types of lipid comprises N1,N3,N5-tris(3-(didodecylamino)propyl)benzene-1,3,5-tricarboxamide (TT3); and
(ii) a self-amplifying viral replicon RNA comprising a sequence that encodes an interleukin (IL)-12 molecule;

and
wherein the IL-12 molecule is expressed by the self-amplifying viral replicon RNA.

[0006] The invention also provides a pharmaceutical composition comprising the synthetic oncolytic virus of the invention and a pharmaceutically acceptable carrier.

[0007] The invention also provides the synthetic oncolytic virus of the invention or the pharmaceutical composition of the invention for use in treating a cancer in a subject.

[0008] Any references in the description to methods of treatment which comprise use of a synthetic oncolytic virus of the invention or of a pharmaceutical composition of the invention and which constitute a method for treatment of the human or animal body by surgery or therapy or a diagnostic method practised on the human or animal body are to be interpreted as references to the synthetic oncolytic virus or pharmaceutical composition for use in those methods.

SUMMARY OF THE DISCLOSURE

[0009] The present disclosure is based, at least in part, on the unexpected discovery that upon injection at a tumor site, a synthetic oncolytic virus successfully triggers anti-cancer immune response of local tumors and enables systemic immunity against distal tumors.

[0010] Accordingly, one aspect of the present disclosure provides a synthetic oncolytic virus comprising a lipid nanoparticle comprising one or more types of lipid and a self-amplifying replicon RNA comprising a sequence that encodes an interleukin (IL)-12 molecule. The lipid nanoparticle is capable of triggering immunogenic cell death. The IL-12 molecule is expressed by the self-amplifying replicon RNA.

[0011] The synthetic oncolytic virus comprises a lipid nanoparticle. The lipid nanoparticle comprises one or more types of lipids. In some instances, the one or more types of lipids comprises a cationic lipid. The lipid nanoparticle according to the invention comprises the cationic lipid N1,N3,N5-tris(3-(didodecylamino)propyl)benzene-1,3,5-tricarboxamide (TT3). In some instances, the lipid nanoparticle comprises TT3, 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, and C14-PEG2000.

[0012] Further, the synthetic oncolytic virus comprises a self-amplifying replicon RNA. In some instances, the self-amplicon RNA is derived from an alphavirus or other Group IV viruses (positive single strand RNA viruses, such as a hepatitis C virus (HCV)). In some instances, the alphavirus can be Venezuela Equine Encephalitis virus, Semliki Forest virus, or Sindbis virus.

[0013] The self-amplifying replicon RNA comprises a sequence encoding an IL-12 molecule. In some instances, the sequence that encodes the IL-12 molecule is located in a subgenomic region of the self-amplifying replicon RNA.

[0014] In some instances, the self-amplifying replicon RNA comprises a nucleotide sequence that is at least 90%

identical of the wild type (WT) replicon RNA having a sequence of SEQ ID NO: 1. In some instances, the self-amplifying replicon RNA is not identical to SEQ ID NO: 1 and is capable of expressing the IL-12 molecule at a higher level compared to the self-amplifying replicon RNA comprising SEQ ID NO: 1. In some instances, replicon RNA that is capable of expressing the IL-12 molecule at a higher level comprises a point mutations of G3936C and/or A4758G of SEQ ID NO: 1.

[0015] In some instances, the self-amplifying replicon RNA further comprises a serum albumin coding sequence. In some instances, the self-amplifying replicon RNA further comprises a Lumican coding sequence.

[0016] The self-amplifying replicon RNA comprises a sequence encoding a IL-12 molecule. In some instances, the IL-12 molecule is selected from the group consisting of IL-12, an IL-12 subunit, or a mutant IL-12 molecule that retains the immunomodulatory function. In some further instances, the IL-12 molecule comprises IL12α and/or IL12β subunits.

[0017] In some instances, the lipid nanoparticle has a diameter of about 100-120 nm. In some instances the lipid nanoparticle has a zeta potential of about 3-6 mv.

[0018] In some instances, the lipid and the self-amplifying replicon RNA have a mass ratio of about 1:2 to 2:1.

[0019] The present disclosure, at least in part, relates to a pharmaceutical composition, comprising the synthetic oncolytic virus and a pharmaceutically acceptable carrier.

[0020] In some instances, pharmaceutical composition is formulated for intratumoral injection.

[0021] The present disclosure, at least in part, relates to a method for treating cancer in a subject in need thereof, comprising administering to the subject an effective amount of the synthetic oncolytic virus of any or the synthetic oncolytic virus-containing pharmaceutical composition.

[0022] In some instances, the subject is a human patient having or suspected of having a cancer. Exemplary target cancers include, but are not limited to melanoma, breast cancer and colon cancer.

[0023] In some instances, the pharmaceutical composition is administered to the subject in a single dose. In some instances, the pharmaceutical composition is administered to the subject by intratumoral injection.

[0024] Also within the present disclosure are any of the synthetic oncolytic virus-containing pharmaceutical compositions described herein for use in treating any of the target diseases disclosed herein (e.g., cancer), as well as pharmaceutical compositions comprising the synthetic oncolytic oligonucleotide for use in manufacturing a medicament for cancer treatment.

[0025] The details of one or more instances of the disclosure are set forth in the description below. Features or advantages of the present invention will be apparent from the following drawings and detailed description, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026]

FIGs. 1A-1H are charts showing the effects of B16F 10 cells transfected with DOTAP, lipofectamine (Lipo), TT3 nanoparticle (TT3), mutant replicon RNA (RNA), DOTAP, Lipo, and TT3 nanoparticle encapsulate mutant replicon RNA (DOTAP-mtRep, Lipo-mtRep, and TT3-mtRep), electroporation (Electro), and electroporation with mutant replicon RNA (Electro-mtRep). FIG. 1A is a graph showing B16F10 cell viability 3 days post transfection. FIG. 1B is a graph showing GFP expression in B16F10 cells transfected with lipid nanoparticle encapsulated with mutant or dead replicon RNA (deRep). FIG. 1C is a graph showing percentages of PI+ Annexin V+ dead B16F10 cells transfected with lipid nanoparticles or with lipid nanoparticles encapsulated with replicon RNA. Immunogenic cell death induced by lipid nanoparticles or with lipid nanoparticles encapsulated with replicon RNA, was shown by percent of calreticulin + (CRT+) cells (FIG. 1D), extracellular ATP (FIG. 1E) and HMGB1 release (FIG. 1F). FIG. 1G shows the diameter of the lipid nanoparticles and lipid nanoparticle loaded with mutant replicon RNAs. FIG. 1H shows the zeta potential of the lipid nanoparticles and lipid nanoparticle loaded with mutant replicon RNAs.

FIGs. 2A-2J are charts showing mtRep and deRep trigger TLR3 signaling and induce ISGF3 complex to necrotic cell death. LNP-replicon RNA trigger TLR3 signaling (FIG. 2B and FIG. 2E) and activates the expression of interferon stimulated genes Stat 1, Stat2, IRF9, IRF3 and cGAS (FIGs. 2F-2J), but does not activate TLR2 (FIG. 2A), TLR7 (FIG. 2C) or TLR9 (FIG. 2D).

FIG. 3A-3K are charts showing TT3-mtRep recruits immune cells and regresses tumor growth. FIG. 3A shows mtRep recruits more Ly6clo Ly6G+ granulocytes 3 days post injection. FIG. 3B shows the percentages of mCherry+ cells in CD45+ and CD45- cells in tumors injected with TT3-mtRep 3 days post injection. FIG. 3C shows the expression of ISGF3 complex (Stat1/Stat2/IRF9) as well as IRF3 and cGAS at 1 day post TT3-mtRep injection. FIGs. 3D-3F show the immune cell infiltration (granulocytes, M-MDSC, monocytes, macrophages, CD4 T, CD8 T, NK, NKT, conventional DC1 (cDC1), and conventional DC2 (cDC2)) into the tumor at day 1 (FIG. 3D), and at day 3 (FIG. 3E) post one injection of TT3-mtRep, and at day 1 post 3 sequential injection (FIG. 3F) of TT3-mtRep. FIGs. 3G-3H show TT3-mtRep induces more cell death (FIG. 3G), more immune cell infiltration (FIG. 3H), reduced tumor weight (FIG. 3I), tumor area (FIG. 3J) and increased expression of CCL5 (FIG. 3K) at one day after 3 sequential injection of TT3-mtRep.

FIGs. 4A-4G are charts showing TT3-mtRep encoding IL12-MSA or IL12-MSA-Lumican effectively modulates tumor microenvironments and immune cell infiltration. FIGs. 4A and 4B show quantification of IFNα2 by ELISA in tumor (FIG. 4A) and in serum (FIG. 4B) one day and three days post TT3-mtRep injection. FIG. 4C shows B16F10 cells transfected with mtRep-IL12-MSA and mtRep-IL12-MSA-Lumican can produce IL-12. FIG. 4D shows IL-12 level in tumor one day and three days post single injection of TT3-mtRep, TT3-mtRep-IL12-MSA, or TT3-mtRep-IL12-MSA-lumican. FIG. 4E-4F shows immune cell infiltration in tumor one day (FIG. 4E) and three days (FIG. 4F) post single injection of TT3-mtRep, TT3-mtRep-IL12-MSA, or TT3-mtRep-IL12-MSA-lumican. FIG. 4G shows numbers of conventional DC1 (cDC1) in tumor draining lymph node one day and three days post single injection of TT3-mtRep, TT3-mtRep-IL12-MSA, or TT3-mtRep-IL12-MSA-lumican.

FIGs. 5A-5E are charts showing *in vivo* synergistic anti-cancer effects of immunomodulatory IL12 and immunogenic cell death induced by TT3-mtRep. The synergistic anti-cancer effects were evaluated by changes of body weight (FIG. 5A), IFNr in serum (FIG. 5B), tumor area (FIG. 5C), and survival curve (FIG. 5D). FIG. 5E shows that TT3-mtRep-IL12 treatment can prevent the recurrence of tumor in cured mice.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0027] The present disclosure is based, at least in part, on the unexpected discovery that upon injection at a tumor site, a synthetic oncolytic virus comprising a lipid nanoparticle comprising one or more types of lipid and a self-amplifying replicon RNA comprising a sequence that encodes an IL-12 molecule successfully triggers anti-cancer immune response of local tumors and enables systemic immunity against distal tumors.

I. Synthetic Oncolytic Virus

[0028] The present disclosure, at least in part, relates to a synthetic oncolytic virus. As used herein, the term "synthetic" refers to a non-natural or engineered oncolytic virus as disclosed herein, which includes a lipid nanoparticle and a self-amplifying replicon RNA comprising a sequence that encodes an IL-12 molecule.

[0029] In some aspects, the present disclosure relates to utilizing lipid nanoparticles (LNPs), which are capable of inducing immunogenic cell death by themselves, to facilitate the delivery of biologically active agent (e.g., a self-amplifying replicon RNA encoding an IL-12 molecule) into the tumor cells.

(i) Lipid Nanoparticle

[0030] The present disclosure, at least in part, relates to the delivery of biologically active molecules to cells using lipid nanoparticles. Specifically, described herein is a synthetic oncolytic virus comprising IL-12 expressing self-amplifying replicon RNA encapsulated by lipid nanoparticles, the composition thereof, and methods of using the synthetic oncolytic virus, and the composition thereof to treat a subject having cancer or suspected of having cancer.

[0031] A lipid nanoparticle (LNPs), as used herein, refers to vesicle, such as a spherical vesicle, having a contiguous lipid bilayer. Lipid nanoparticles can be used in methods by which pharmaceutical therapies are delivered to targeted locations. Non-limiting examples of LNPs include liposomes, bolaamphiphiles, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and monolayer membrane structures (e.g., archaeosomes and micelles).

[0032] The lipid nanoparticle, as used herein, comprises one or more types of lipids. A lipid, as used herein, refers to a group of organic compounds that include, but are not limited to, esters of fatty acids and in some instances are characterized by being insoluble in water, but soluble in many organic solvents. They are usually divided into at least three classes: (1) "simple lipids" which include fats and oils as well as waxes; (2) "compound lipids" which include phospholipids and glycolipids; (3) "derived lipids" such as steroids. Non-limiting examples of lipids include triglycerides (e.g. tristearin), diglycerides (e.g. glycerol bahenate), monoglycerides (e.g. glycerol monostearate), fatty acids (e.g. stearic acid), steroids (e.g. cholesterol), and waxes (e.g. cetyl palmitate). In some instances, the one or more types of lipids in the LNP, comprises a cationic lipid.

[0033] A cationic lipid, as used herein, refers to any of a number of lipid species that carry a net positive charge at a selected pH, such as physiological pH. Such lipids include, but are not limited to N1,N3,N5-tris(3-(didodecylamino)propyl) benzene-1,3,5-tricarboxamide (TT3), N-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP); lipofectamine; 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), dioctadecyldimethylammonium (DODMA), Distearyldimethylammonium (DSDMA), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC); N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA); N,N-distearyl-N,N-dimethylammonium bromide (DDAB); 3-(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol (DC-Chol) and N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE).

[0034] In the lipid nanoparticles according to the invention, the cationic lipid is TT3. TT3, as used herein, is capable of forming lipid nanoparticles for delivery of various biologic active agents into the cells. In addition, the present disclosure

also demonstrates that an unloaded TT3-LNP can induce immunogenic cell death (ICD) in cancer cells *in vivo* and *in vitro.* Immunogenic cell death, as described herein, refers to a form of cell death that can induce an effective immune response through activation of dendritic cells (DCs) and consequent activation of specific T cell response. In some instances, the cells that undergo immunogenic cell death (ICD) are tumor cells. Immunogenic tumor cell death can trigger an effective anti-tumor immune response. In some instances, the synthetic oncolytic virus comprises TT3-LNP encapsulating a self-amplifying replicon RNA encoding only a reporter gene (TT3-LNP-replicon RNA). The self-amplifying replicon RNA can work synergistically with the TT3-LNP to induce higher level of ICD in tumor cells compared to TT3-LNP alone. In other instances, the synthetic oncolytic virus comprises a TT3-LNP encapsulating a self-amplifying replicon RNA encoding an IL-12 molecule. IL-12, which is an immunoregulatory cytokine, elicits potent immune response against the local tumor. Moreover, the combination of TT3-LNP, self-amplifying replicon RNA and IL-12 expression, not only is effective in synergistic inhibition of tumor cells on site, but also elicits a systemic anti-tumor immune response to kill distal tumor cells and prevent the recurrence of tumors.

[0035] In some instances, the cationic lipid is DOTAP. DOTAP, as used herein, is also capable of forming lipid nanoparticles. DOTAP can be used for the highly efficient transfection of DNA including yeast artificial chromosomes (YACs) into eukaryotic cells for transient or stable gene expression, and is also suitable for the efficient transfer of other negatively charged molecules, such as RNA, oligonucleotides, nucleotides, ribonucleo-protein (RNP) complexes, and proteins into research samples of mammalian cells.

[0036] In other instances, the cationic lipid is lipofectamine. Lipofectamine, as used herein, is a common transfection reagent, produced and sold by Invitrogen, used in molecular and cellular biology. It is used to increase the transfection efficiency of RNA (including mRNA and siRNA) or plasmid DNA into in vitro cell cultures by lipofection. Lipofectamine contains lipid subunits that can form liposomes or lipid nanoparticles in an aqueous environment, which entrap the transfection payload, e.g. self-amplifying replicon RNA. The RNA-containing liposomes (positively charged on their surface) can fuse with the negatively charged plasma membrane of living cells, due to the neutral co-lipid mediating fusion of the liposome with the cell membrane, allowing nucleic acid cargo molecules to cross into the cytoplasm for replication or expression.

[0037] In some instances, LNPs are composed primarily of cationic lipids along with other lipid ingredients. These typically include other lipid molecules belonging but not limited to the phosphatidylcholine (PC) class (e.g., 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), and 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), sterols (e.g., cholesterol) and Polyethylene glycol (PEG)-lipid conjugates (e.g., 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(polyethylene glycol)-2000 (DSPE-PEG2000) and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000 (C14-PEG2000). Table 1 shows the formulation of two LNPs, TT3-LNP and DOTAP-LNP.

Table 1:

DOTAP-LNP	DOTAP	DSPC	Cholesterol	DSPE-PEG2000
Molar ratio	40	10	48	2
TT3-LNP	TT3	DOPE	Cholesterol	C14-PEG2000
Molar Ratio	20	30	40	0.75

[0038] The synthetic oncolytic virus of the invention comprises one or more types of lipid, wherein the one or more types of lipid comprises N1,N3,N5-tris(3-(didodecylamino)propyl)benzene-1,3,5-tricarboxamide (TT3).

[0039] Particle size of lipid nanoparticles can affect drug release rate, bio-distribution, mucoadhesion, cellular uptake of water and buffer exchange to the interior of the nanoparticles, and protein diffusion. In some instances, the diameter of the LNPs ranges from 30 to 150 nm. In some instances, the diameter of the LNPs ranges from 100-120 nm. In some instances, the diameter of the LNPs can be 30 nm, 40 nm, 50 nm, 60 nm, 70 nm, 80 nm, 90 nm, 100 nm, 101 nm, 102 nm, 103 nm, 104 nm, 105 nm, 106 nm, 107 nm, 108 nm, 109 nm, 110 nm, 111 nm, 112 nm, 113 nm, 114 nm, 115 nm, 116 nm, 117 nm, 118 nm, 119 nm, or 120 nm.

[0040] Zeta potential is a measure of the effective electric charge on the lipid nanoparticle surface. The magnitude of the zeta potential provides information about particle stability. In some instances, the zeta potential of the LNPs ranges from -10 millivolts (mv) to 25 mv. In some instances, the zeta potential of the LNPs ranges from 3-6 mv. In some instances, the zeta potential of the LNPs can be 3 mv, 3.1 mv, 3.2 mv, 3.3 mv, 3.4 mv, 3.5mv, 3.6 mv, 3.7 mv, 3.8 mv, 3.9 mv, 4 mv, 4.1 mv, 4.2 mv, 4.3 mv, 4.4 mv, 4.5mv, 4.6 mv, 4.7 mv, 4.8 mv, 4.9 mv, 5 mv, 5.1 mv, 5.2 mv, 5.3 mv, 5.4 mv, 5.5mv, 5.6 mv, 5.7 mv, 5.8 mv, 5.9 mv, and 6 mv.

[0041] The present disclosure, at least in part, is related to encapsulating replicon RNA with the lipid nanoparticles. In some instances, the mass ratio between the LNPs and the replicon RNA ranges from 1:2 to 2:1. In some instances, the mass ratio between the LNPs and the replicon RNA can be 1:2, 1:1.5, 1:1.2, 1:1, 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1 and 2:1. In some instances, the mass ratio between the LNPs and the replicon RNA can be 1:1.

(ii) Self-amplifying replicon RNA

[0042] In the present disclosure, a self-amplifying replicon RNA encoding IL-12 molecule under the subgenomic promoter, in place of the structural proteins required for virus replication, are of substantial interest in cancer immunotherapy. As used herein, the term "self-amplifying replicon RNA" refers to a self-replicating genetic element comprised a RNA that replicates from one origin of replication. The terms "replicon RNA" and "self-amplifying replicon RNA" are used interchangeably herein. In some instances, the self-amplifying replicon RNA is a viral replicon.

[0043] The synthetic oncolytic virus of the invention comprises a self-amplifying viral replicon RNA comprising a sequence that encodes an interleukin (IL)-12 molecule wherein the IL-12 molecule is expressed by the self-amplifying viral replicon RNA.A virus is a small pathogen that is only capable of replication inside a living host cell (e.g., prokaryotic and eukaryotic cells). Outside of living cells, viruses exist as independent particles (e.g., viral particles or virions), which comprise genetic material in the form of DNA or RNA, the latter of which can be single-stranded or double-stranded. Viruses with DNA are referred to as DNA viruses, and viruses with RNA are referred to as RNA viruses. In some cases, the virus comprises nucleic acid-associated proteins and the combination of the virus and nucleic acid-associated proteins is referred to as nucleoprotein. In addition to the genetic material, viruses have a single or double protein coat, also known as a capsid, which facilitates attachment of the virus to a living host cell's receptors during infection and protects the genetic material of the virus from enzymatic degradation. The combination of nucleoprotein and the capsid is referred to as a nucleocapsid. In some cases, viruses have a lipid bilayer envelope, studded with virus-coded, glycosylated (trans-) membrane-associated proteins. Once a virus has infected a living host cell, the virus is dependent on the living host cell to supply the machinery for its replication, and propagation thereafter. The viral genome codes for some structural proteins and non-structural regulatory proteins.

[0044] As used herein, the term "subgenome" or "subgenomic" refers to a smaller section of the whole replicon genome. Accordingly, subgenomic transcription, as used herein, refers to the transcription of one or more genes in the replicon genome but not all the genes constituting the replicon genome. In one instance, subgenomic transcription refers to transcription of the genes of experimental or therapeutic interest, which are described elsewhere herein.

[0045] The term "structural protein," as used in the context of viruses herein, refers to proteins that constitute the structural components of mature assembled virus particles or virions. Non-limiting examples of such structural proteins include nucleocapsid core proteins (*e.g.,* gag proteins), enzymes packaged within the virus particle (*e.g.,* pol proteins), and membrane components (*e.g*., env proteins). In contrast, the term "non-structural protein," as used in the context of viruses herein, refer to proteins that are expressed within the host cell but do not constitute structural components of the virus particle or virion. Some of the roles of non-structural proteins include, but are not limited to, replicon formation, immunomodulation, and transactivation of structural protein genes.

[0046] In some embodiments, the self-amplifying replicon RNA is derived from an alphavirus. Distinct from host mRNA, alphavirus replicon RNAs encode a set of four nonstructural proteins (nsPs 1-4) that are responsible both for genome replication and, when engineered to include genes encoding non-virus products, such as IL-12 molecules, provide for transcription of such non-viral products under the subgenomic promoter. Alphaviruses are part of the Group IV Togaviridae family of viruses, possess a positive sense, single-stranded RNA genome, and are characterized by an icosahedral nucleocapsid. Other non-limiting examples of Group IV viruses can be Astroviridae, Caliciviridae, Coronaviridae, Flaviviridae, Picornaviridae, Arteriviridae, and Togaviridae. The alphavirus genus includes 26 enveloped viruses that infect eukaryotes. Alphaviruses have a broad host range and are transmitted by mosquitos and hematophagous arthropods. Non-limiting examples of alphaviruses include Venezuelan equine encephalitis virus (VEE), Semliki Forest virus (SF), Sindbis virus (SIN),Eastern Equine Encephalitis virus (EEE), Western equine encephalitis virus (WEE), Everglades virus (EVE), Mucambo virus (MUC), Pixuna virus (PIX), Semliki Forest virus (SF), Middelburg virus (MID), Chikungunya virus (CHIK), O'Nyong-Nyong virus (ONN), Ross River virus (RR), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAG), Bebaru virus (BEB), Mayaro virus (MAY), Una virus (UNA), Aura virus (AURA), Babanki virus (BAB), Highlands J virus (HJ), and Fort Morgan virus (FM).

[0047] In the present disclosure, at least in part, the alphavirus replicon is a VEE alphavirus replicon. The VEE virus is a viral pathogen typically carried by mosquitos that causes VEE or encephalomyelitis predominately in equine species. Humans, however, may also contract VEE, and people with weakened immune systems are especially at risk of having severe complications if infected with VEE. The virion of VEE is spherical and possesses a lipid membrane with glycoprotein surface proteins spread around the outer surface. Typically, VEE has a genome of approximately 11.45 kb, excluding the 5'-terminal cap and 3'-terminal poly(A) tract, and comprises 4 nonstructural proteins (nsPs) and 5 structural proteins. The non-structural proteins include nsP1, nsP2, nsP3, and nsP4, while the structural region encodes proteins C, E3, E2, 6K, and E1. In some instances, the self-amplifying replicon RNA is a WT replicon RNA derived from VEE. The sequence of the VEE virus WT replicon RNA is set forth in SEQ ID NO: 1:

ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAAATGGAGAAAGTTCACGTT
GACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGGAGCTTCCCGCAGTTTGAGGT
AGAAGCCAAGCAGGTCACTGATAATGACCATGCTAATGCCAGAGCGTTTTCGCATCTGGCTT
CAAAACTGATCGAAACGGAGGTGGACCCATCCGACACGATCCTTGACATTGGAAGTGCGCCC
GCCCGCAGAATGTATTCTAAGCACAAGTATCATTGTATCTGTCCGATGAGATGTGCGGAAGA
TCCGGACAGATTGTATAAGTATGCAACTAAGCTGAAGAAAAACTGTAAGGAAATAACTGATA
AGGAATTGGACAAGAAAATGAAGGAGCTGGCCGCCGTCATGAGCGACCCTGACCTGGAAACT
GAGACTATGTGCCTCCACGACGACGAGTCGTGTCGCTACGAAGGGCAAGTCGCTGTTTACCA
GGATGTATACGCGGTTGACGGACCGACAAGTCTCTATCACCAAGCCAATAAGGGAGTTAGAG
TCGCCTACTGGATAGGCTTTGACACCACCCCTTTTATGTTTAAGAACTTGGCTGGAGCATAT
CCATCATACTCTACCAACTGGGCCGACGAAACCGTGTTAACGGCTCGTAACATAGGCCTATG
CAGCTCTGACGTTATGGAGCGGTCACGTAGAGGGATGTCCATTCTTAGAAAGAAGTATTTGA
AACCATCCAACAATGTTCTATTCTCTGTTGGCTCGACCATCTACCACGAGAAGAGGGACTTA
CTGAGGAGCTGGCACCTGCCGTCTGTATTTCACTTACGTGGCAAGCAAAATTACACATGTCG
GTGTGAGACTATAGTTAGTTGCGACGGGTACGTCGTTAAAAGAATAGCTATCAGTCCAGGCC
TGTATGGGAAGCCTTCAGGCTATGCTGCTACGATGCACCGCGAGGGATTCTTGTGCTGCAAA
GTGACAGACACATTGAACGGGGAGAGGGTCTCTTTTCCCGTGTGCACGTATGTGCCAGCTAC
ATTGTGTGACCAAATGACTGGCATACTGGCAACAGATGTCAGTGCGGACGACGCGCAAAAAC
TGCTGGTTGGGCTCAACCAGCGTATAGTCGTCAACGGTCGCACCCAGAGAAACACCAATACC
ATGAAAAATTACCTTTTGCCCGTAGTGGCCCAGGCATTTGCTAGGTGGGCAAAGGAATATAA
GGAAGATCAAGAAGATGAAAGGCCACTAGGACTACGAGATAGACAGTTAGTCATGGGGTGTT
GTTGGGCTTTTAGAAGGCACAAGATAACATCTATTTATAAGCGCCCGGATACCCAAACCATC
ATCAAAGTGAACAGCGATTTCCACTCATTCGTGCTGCCCAGGATAGGCAGTAACACATTGGA
GATCGGGCTGAGAACAAGAATCAGGAAAATGTTAGAGGAGCACAAGGAGCCGTCACCTCTCA
TTACCGCCGAGGACGTACAAGAAGCTAAGTGCGCAGCCGATGAGGCTAAGGAGGTGCGTGAA
GCCGAGGAGTTGCGCGCAGCTCTACCACCTTTGGCAGCTGATGTTGAGGAGCCCACTCTGGA
GGCAGACGTCGACTTGATGTTACAAGAGGCTGGGGCCGGCTCAGTGGAGACACCTCGTGGCT
TGATAAAGGTTACCAGCTACGATGGCGAGGACAAGATCGGCTCTTACGCTGTGCTTTCTCCG
CAGGCTGTACTCAAGAGTGAAAAATTATCTTGCATCCACCCTCTCGCTGAACAAGTCATAGT
GATAACACACTCTGGCCGAAAAGGGCGTTATGCCGTGGAACCATACCATGGTAAAGTAGTGG
TGCCAGAGGGACATGCAATACCCGTCCAGGACTTTCAAGCTCTGAGTGAAAGTGCCACCATT
GTGTACAACGAACGTGAGTTCGTAAACAGGTACCTGCACCATATTGCCACACATGGAGGAGC
GCTGAACACTGATGAAGAATATTACAAAACTGTCAAGCCCAGCGAGCACGACGGCGAATACC
TGTACGACATCGACAGGAAACAGTGCGTCAAGAAAGAACTAGTCACTGGGCTAGGGCTCACA
GGCGAGCTGGTGGATCCTCCCTTCCATGAATTCGCCTACGAGAGTCTGAGAACACGACCAGC
CGCTCCTTACCAAGTACCAACCATAGGGGTGTATGGCGTGCCAGGATCAGGCAAGTCTGGCA
TCATTAAAAGCGCAGTCACCAAAAAAGATCTAGTGGTGAGCGCCAAGAAAGAAAACTGTGCA
GAAATTATAAGGGACGTCAAGAAAATGAAAGGGCTGGACGTCAATGCCAGAACTGTGGACTC
AGTGCTCTTGAATGGATGCAAACACCCCGTAGAGACCCTGTATATTGACGAAGCTTTTGCTT
GTCATGCAGGTACTCTCAGAGCGCTCATAGCCATTATAAGACCTAAAAAGGCAGTGCTCTGC
GGGGATCCCAAACAGTGCGGTTTTTTTAACATGATGTGCCTGAAAGTGCATTTTAACCACGA

GATTTGCACACAAGTCTTCCACAAAAGCATCTCTCGCCGTTGCACTAAATCTGTGACTTCGG
TCGTCTCAACCTTGTTTTACGACAAAAAAATGAGAACGACGAATCCGAAAGAGACTAAGATT
GTGATTGACACTACCGGCAGTACCAAACCTAAGCAGGACGATCTCATTCTCACTTGTTTCAG
AGGGTGGGTGAAGCAGTTGCAAATAGATTACAAAGGCAACGAAATAATGACGGCAGCTGCCT
CTCAAGGGCTGACCCGTAAAGGTGTGTATGCCGTTCGGTACAAGGTGAATGAAAATCCTCTG
TACGCACCCACCTCAGAACATGTGAACGTCCTACTGACCCGCACGGAGGACCGCATCGTGTG
GAAAACACTAGCCGGCGACCCATGGATAAAAACACTGACTGCCAAGTACCCTGGGAATTTCA
CTGCCACGATAGAGGAGTGGCAAGCAGAGCATGATGCCATCATGAGGCACATCTTGGAGAGA
CCGGACCCTACCGACGTCTTCCAGAATAAGGCAAACGTGTGTTGGGCCAAGGCTTTAGTGCC
GGTGCTGAAGACCGCTGGCATAGACATGACCACTGAACAATGGAACACTGTGGATTATTTTG
AAACGGACAAAGCTCACTCAGCAGAGATAGTATTGAACCAACTATGCGTGAGGTTCTTTGGA
CTCGATCTGGACTCCGGTCTATTTTCTGCACCCACTGTTCCGTTATCCATTAGGAATAATCA
CTGGGATAACTCCCCGTCGCCTAACATGTACGGGCTGAATAAAGAAGTGGTCCGTCAGCTCT
CTCGCAGGTACCCACAACTGCCTCGGGCAGTTGCCACTGGAAGAGTCTATGACATGAACACT
GGTACACTGCGCAATTATGATCCGCGCATAAACCTAGTACCTGTAAACAGAAGACTGCCTCA
TGCTTTAGTCCTCCACCATAATGAACACCCACAGAGTGACTTTTCTTCATTCGTCAGCAAAT
TGAAGGGCAGAACTGTCCTGGTGGTCGGGGAAAAGTTGTCCGTCCCAGGCAAAATGGTTGAC
TGGTTGTCAGACCGGCCTGAGGCTACCTTCAGAGCTCGGCTGGATTTAGGCATCCCAGGTGA
TGTGCCCAAATATGACATAATATTTGTTAATGTGAGGACCCCATATAAATACCATCACTATC
AGCAGTGTGAAGACCATGCCATTAAGCTTAGCATGTTGACCAAGAAAGCTTGTCTGCATCTG
AATCCCGGCGGAACCTGTGTCAGCATAGGTTATGGTTACGCTGACAGGGCCAGCGAAAGCAT
CATTGGTGCTATAGCGCGGCAGTTCAAGTTTTCCCGGGTATGCAAACCGAAATCCTCACTTG
AAGAGACGGAAGTTCTGTTTGTATTCATTGGGTACGATCGCAAGGCCCGTACGCACAATTCT
TACAAGCTTTCATCAACCTTGACCAACATTTATACAGGTTCCAGACTCCACGAAGCCGGATG
TGCACCCTCATATCATGTGGTGCGAGGGGATATTGCCACGGCCACCGAAGGAGTGATTATAA
ATGCTGCTAACAGCAAAGGACAACCTGGCGGAGGGGTGTGCGGAGCGCTGTATAAGAAATTC
CCGGAAAGCTTCGATTTACAGCCGATCGAAGTAGGAAAAGCGCGACTGGTCAAAGGTGCAGC
TAAACATATCATTCATGCCGTAGGACCAAACTTCAACAAAGTTTCGGAGGTTGAAGGTGACA
AACAGTTGGCAGAGGCTTATGAGTCCATCGCTAAGATTGTCAACGATAACAATTACAAGTCA
GTAGCGATTCCACTGTTGTCCACCGGCATCTTTTCCGGGAACAAAGATCGACTAACCCAATC
ATTGAACCATTTGCTGACAGCTTTAGACACCACTGATGCAGATGTAGCCATATACTGCAGGG
ACAAGAAATGGGAAATGACTCTCAAGGAAGCAGTGGCTAGGAGAGAAGCAGTGGAGGAGATA
TGCATATCCGACGACTCTTCAGTGACAGAACCTGATGCAGAGCTGGTGAGGGTGCATCCGAA
GAGTTCTTTGGCTGGAAGGAAGGGCTACAGCACAAGCGATGGCAAAACTTTCTCATATTTGG
AAGGGACCAAGTTTCACCAGGCGGCCAAGGATATAGCAGAAATTAATGCCATGTGGCCCGTT
GCAACGGAGGCCAATGAGCAGGTATGCATGTATATCCTCGGAGAAAGCATGAGCAGTATTAG
GTCGAAATGCCCCGTCGAAGAGTCGGAAGCCTCCACACCACCTAGCACGCTGCCTTGCTTGT
GCATCCATGCCATGACTCCAGAAAGAGTACAGCGCCTAAAAGCCTCACGTCCAGAACAAATT
ACTGTGTGCTCATCCTTTCCATTGCCGAAGTATAGAATCACTGGTGTGCAGAAGATCCAATG
CTCCCAGCCTATATTGTTCTCACCGAAAGTGCCTGCGTATATTCATCCAAGGAAGTATCTCG
TGGAAACACCACCGGTAGACGAGACTCCGGAGCCATCGGCAGAGAACCAATCCACAGAGGGG
ACACCTGAACAACCACCACTTATAACCGAGGATGAGACCAGGACTAGAACGCCTGAGCCGAT
CATCATCGAAGAGGAAGAAGAGGATAGCATAAGTTTGCTGTCAGATGGCCCGACCCACCAGG
TGCTGCAAGTCGAGGCAGACATTCACGGGCCGCCCTCTGTATCTAGCTCATCCTGGTCCATT
CCTCATGCATCCGACTTTGATGTGGACAGTTTATCCATACTTGACACCCTGGAGGGAGCTAG
CGTGACCAGCGGGGCAACGTCAGCCGAGACTAACTCTTACTTCGCAAAGAGTATGGAGTTTC
TGGCGCGACCGGTGCCTGCGCCTCGAACAGTATTCAGGAACCCTCCACATCCCGCTCCGCGC
ACAAGAACACCGTCACTTGCACCCAGCAGGGCCTGCTCGAGAACCAGCCTAGTTTCCACCCC
GCCAGGCGTGAATAGGGTGATCACTAGAGAGGAGCTCGAGGCGCTTACCCCGTCACGCACTC
CTAGCAGGTCGGTCTCGAGAACCAGCCTGGTCTCCAACCCGCCAGGCGTAAATAGGGTGATT
ACAAGAGAGGAGTTTGAGGCGTTCGTAGCACAACAACAATGACGGTTTGATGCGGGTGCATA
CATCTTTTCCTCCGACACCGGTCAAGGGCATTTACAACAAAAATCAGTAAGGCAAACGGTGC

TATCCGAAGTGGTGTTGGAGAGGACCGAATTGGAGATTTCGTATGCCCCGCGCCTCGACCAA
GAAAAAGAAGAATTACTACGCAAGAAATTACAGTTAAATCCCACACCTGCTAACAGAAGCAG
ATACCAGTCCAGGAAGGTGGAGAACATGAAAGCCATAACAGCTAGACGTATTCTGCAAGGCC
TAGGGCATTATTTGAAGGCAGAAGGAAAAGTGGAGTGCTACCGAACCCTGCATCCTGTTCCT
TTGTATTCATCTAGTGTGAACCGTGCCTTTTCAAGCCCCAAGGTCGCAGTGGAAGCCTGTAA
CGCCATGTTGAAAGAGAACTTTCCGACTGTGGCTTCTTACTGTATTATTCCAGAGTACGATG
CCTATTTGGACATGGTTGACGGAGCTTCATGCTGCTTAGACACTGCCAGTTTTTGCCCTGCA
AAGCTGCGCAGCTTTCCAAAGAAACACTCCTATTTGGAACCCACAATACGATCGGCAGTGCC
TTCAGCGATCCAGAACACGCTCCAGAACGTCCTGGCAGCTGCCACAAAAAGAAATTGCAATG
TCACGCAAATGAGAGAATTGCCCGTATTGGATTCGGCGGCCTTTAATGTGGAATGCTTCAAG
AAATATGCGTGTAATAATGAATATTGGGAAACGTTTAAAGAAAACCCCATCAGGCTTACTGA
AGAAAACGTGGTAAATTACATTACCAAATTAAAAGGACCAAAAGCTGCTGCTCTTTTTGCGA
AGACACATAATTTGAATATGTTGCAGGACATACCAATGGACAGGTTTGTAATGGACTTAAAG
AGAGACGTGAAAGTGACTCCAGGAACAAAACATACTGAAGAACGGCCCAAGGTACAGGTGAT
CCAGGCTGCCGATCCGCTAGCAACAGCGTATCTGTGCGGAATCCACCGAGAGCTGGTTAGGA
GATTAAATGCGGTCCTGCTTCCGAACATTCATACACTGTTTGATATGTCGGCTGAAGACTTT
GACGCTATTATAGCCGAGCACTTCCAGCCTGGGGATTGTGTTCTGGAAACTGACATCGCGTC
GTTTGATAAAAGTGAGGACGACGCCATGGCTCTGACCGCGTTAATGATTCTGGAAGACTTAG
GTGTGGACGCAGAGCTGTTGACGCTGATTGAGGCGGCTTTCGGCGAAATTTCATCAATACAT
TTGCCCACTAAAACTAAATTTAAATTCGGAGCCATGATGAAATCTGGAATGTTCCTCACACT
GTTTGTGAACACAGTCATTAACATTGTAATCGCAAGCAGAGTGTTGAGAGAACGGCTAACCG
GATCACCATGTGCAGCATTCATTGGAGATGACAATATCGTGAAAGGAGTCAAATCGGACAAA
TTAATGGCAGACAGGTGCGCCACCTGGTTGAATATGGAAGTCAAGATTATAGATGCTGTGGT
GGGCGAGAAAGCGCCTTATTTCTGTGGAGGGTTTATTTTGTGTGACTCCGTGACCGGCACAG
CGTGCCGTGTGGCAGACCCCCTAAAAAGGCTGTTTAAGCTTGGCAAACCTCTGGCAGCAGAC
GATGAACATGATGATGACAGGAGAAGGGCATTGCATGAAGAGTCAACACGCTGGAACCGAGT
GGGTATTCTTTCAGAGCTGTGCAAGGCAGTAGAATCAAGGTATGAAACCGTAGGAACTTCCA
TCATAGTTATGGCCATGACTACTCTAGCTAGCAGTGTTAAATCATTCAGCTACCTGAGAGGG
GCCCCTATAACTCTCTACGGCTAACCTGAATGGACTACGACATAGTCTAGTCCGCCAAGTCT
AGCATATGGGCGCGCCCTCAGCATCGATTGAATTGGCCACCATGGTGAGCAAGGGCGAGGAG
GATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAA
CGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCG
CCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAG
TTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCT
GTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGA
CCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGC
ACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTC
CGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGA
AGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAG
CTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACAC
CATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGT
ACAAGTAGGAATTGGCAAGCTGCTTACATAGAACTCGCGGCGATTGGCATGCCGCCTTAAAA
TTTTTATTTTATTTTTCTTTTCTTTTCCGAATCGGATTTTGTTTTTAATATTTCAAAAAAAA
AAAAAAAAAAAAAAAAAACGCGTCGAGGGGAATTAATTCTTGAAGACGAAAGGGCCAGGTGG
CACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATA
TGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGT
ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGT
TTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAG
TGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAA
CGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGA
CGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACT
CACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCC

ATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGA
GCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGG
AGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACA
ACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGA
CTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGT
TTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGG
CCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGA
TGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAG
ACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATC
TAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCA
CTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCG
TAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAA
GAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGT
CCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACC
TCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGG
TTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTG
CACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATT
GAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTC
GGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGT
CGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCC
TATGGAAAAACGCCAGCAACGCGAGCTCTAATACGACTCACTATAG (SEQ ID NO: 1)

[0048] The self-amplifying replicon RNA, as described herein, comprises a nucleotide sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO: 1. In some instances, the self-amplifying replicon RNA is at least 90% identical to SEQ ID NO: 1.

[0049] Self-amplifying RNA (replicon) is a promising new platform for gene therapy, but applications are still limited by short persistence of expression in some cell types and low levels of transgene expression *in vivo.* An *in vitro* evolution synthetic replicon RNA provides a potentially powerful strategy for modifying and enhancing replicon expression both *in vitro* and *in vivo.* Using the method of *in vitro* evolution, mutations were identified in nsP2 and nsP3 of Venezuelan equine encephalitis (VEE) replicon that promoted subgenomic expression in human cells.

[0050] In some instances, the self-amplifying replicon RNA comprises mutations that render the replicon RNA capable of expressing the IL-12 molecule at a higher level compared to the replicon RNA comprising SEQ ID NO: 1. In some instances, the self-amplifying replicon RNA, comprises at least one point mutation in a nucleic acid position 3936 and/or 4758 of WT replicon of SEQ ID NO: 1. In some instances, the self-amplifying replicon RNA, comprises at least one of the following point mutations: guanine to cytosine at position 3936 (G3936C) and adenine to guanine at position 4758 (A4758G) of WT replicon sequence of SEQ ID NO: 1. The G3936C mutation would result in a glycine to arginine change at amino acid residue 1309 (G1309R). The A4758G mutation would result in a serine to glycine change at amino acid residue 1583 (S1583G). In some instances, the sequence of the mutant self-amplifying replicon RNA (mtReplicon RNA) is set forth in SEQ ID NO: 2 with the mutations highlighted (bold, underlined text):

ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAAATGGAGAAAGTTCACGTT
GACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGGAGCTTCCCGCAGTTTGAGGT
AGAAGCCAAGCAGGTCACTGATAATGACCATGCTAATGCCAGAGCGTTTTCGCATCTGGCTT
CAAAACTGATCGAAACGGAGGTGGACCCATCCGACACGATCCTTGACATTGGAAGTGCGCCC
GCCCGCAGAATGTATTCTAAGCACAAGTATCATTGTATCTGTCCGATGAGATGTGCGGAAGA
TCCGGACAGATTGTATAAGTATGCAACTAAGCTGAAGAAAAACTGTAAGGAAATAACTGATA
AGGAATTGGACAAGAAAATGAAGGAGCTGGCCGCCGTCATGAGCGACCCTGACCTGGAAACT
GAGACTATGTGCCTCCACGACGACGAGTCGTGTCGCTACGAAGGGCAAGTCGCTGTTTACCA
GGATGTATACGCGGTTGACGGACCGACAAGTCTCTATCACCAAGCCAATAAGGGAGTTAGAG
TCGCCTACTGGATAGGCTTTGACACCACCCCTTTTATGTTTAAGAACTTGGCTGGAGCATAT
CCATCATACTCTACCAACTGGGCCGACGAAACCGTGTTAACGGCTCGTAACATAGGCCTATG
CAGCTCTGACGTTATGGAGCGGTCACGTAGAGGGATGTCCATTCTTAGAAAGAAGTATTTGA
AACCATCCAACAATGTTCTATTCTCTGTTGGCTCGACCATCTACCACGAGAAGAGGGACTTA
CTGAGGAGCTGGCACCTGCCGTCTGTATTTCACTTACGTGGCAAGCAAAATTACACATGTCG
GTGTGAGACTATAGTTAGTTGCGACGGGTACGTCGTTAAAAGAATAGCTATCAGTCCAGGCC
TGTATGGGAAGCCTTCAGGCTATGCTGCTACGATGCACCGCGAGGGATTCTTGTGCTGCAAA
GTGACAGACACATTGAACGGGGAGAGGGTCTCTTTTCCCGTGTGCACGTATGTGCCAGCTAC
ATTGTGTGACCAAATGACTGGCATACTGGCAACAGATGTCAGTGCGGACGACGCGCAAAAAC
TGCTGGTTGGGCTCAACCAGCGTATAGTCGTCAACGGTCGCACCCAGAGAAACACCAATACC
ATGAAAAATTACCTTTTGCCCGTAGTGGCCCAGGCATTTGCTAGGTGGGCAAAGGAATATAA
GGAAGATCAAGAAGATGAAAGGCCACTAGGACTACGAGATAGACAGTTAGTCATGGGGTGTT
GTTGGGCTTTTAGAAGGCACAAGATAACATCTATTTATAAGCGCCCGGATACCCAAACCATC
ATCAAAGTGAACAGCGATTTCCACTCATTCGTGCTGCCCAGGATAGGCAGTAACACATTGGA
GATCGGGCTGAGAACAAGAATCAGGAAAATGTTAGAGGAGCACAAGGAGCCGTCACCTCTCA
TTACCGCCGAGGACGTACAAGAAGCTAAGTGCGCAGCCGATGAGGCTAAGGAGGTGCGTGAA
GCCGAGGAGTTGCGCGCAGCTCTACCACCTTTGGCAGCTGATGTTGAGGAGCCCACTCTGGA
GGCAGACGTCGACTTGATGTTACAAGAGGCTGGGGCCGGCTCAGTGGAGACACCTCGTGGCT
TGATAAAGGTTACCAGCTACGATGGCGAGGACAAGATCGGCTCTTACGCTGTGCTTTCTCCG
CAGGCTGTACTCAAGAGTGAAAAATTATCTTGCATCCACCCTCTCGCTGAACAAGTCATAGT
GATAACACACTCTGGCCGAAAAGGGCGTTATGCCGTGGAACCATACCATGGTAAAGTAGTGG
TGCCAGAGGGACATGCAATACCCGTCCAGGACTTTCAAGCTCTGAGTGAAAGTGCCACCATT
GTGTACAACGAACGTGAGTTCGTAAACAGGTACCTGCACCATATTGCCACACATGGAGGAGC
GCTGAACACTGATGAAGAATATTACAAAACTGTCAAGCCCAGCGAGCACGACGGCGAATACC
TGTACGACATCGACAGGAAACAGTGCGTCAAGAAAGAACTAGTCACTGGGCTAGGGCTCACA
GGCGAGCTGGTGGATCCTCCCTTCCATGAATTCGCCTACGAGAGTCTGAGAACACGACCAGC
CGCTCCTTACCAAGTACCAACCATAGGGGTGTATGGCGTGCCAGGATCAGGCAAGTCTGGCA
TCATTAAAAGCGCAGTCACCAAAAAAGATCTAGTGGTGAGCGCCAAGAAAGAAAACTGTGCA
GAAATTATAAGGGACGTCAAGAAAATGAAAGGGCTGGACGTCAATGCCAGAACTGTGGACTC
AGTGCTCTTGAATGGATGCAAACACCCCGTAGAGACCCTGTATATTGACGAAGCTTTTGCTT
GTCATGCAGGTACTCTCAGAGCGCTCATAGCCATTATAAGACCTAAAAAGGCAGTGCTCTGC
GGGGATCCCAAACAGTGCGGTTTTTTTAACATGATGTGCCTGAAAGTGCATTTTAACCACGA
GATTTGCACACAAGTCTTCCACAAAAGCATCTCTCGCCGTTGCACTAAATCTGTGACTTCGG
TCGTCTCAACCTTGTTTTACGACAAAAAAATGAGAACGACGAATCCGAAAGAGACTAAGATT
GTGATTGACACTACCGGCAGTACCAAACCTAAGCAGGACGATCTCATTCTCACTTGTTTCAG
AGGGTGGGTGAAGCAGTTGCAAATAGATTACAAAGGCAACGAAATAATGACGGCAGCTGCCT
CTCAAGGGCTGACCCGTAAAGGTGTGTATGCCGTTCGGTACAAGGTGAATGAAAATCCTCTG
TACGCACCCACCTCAGAACATGTGAACGTCCTACTGACCCGCACGGAGGACCGCATCGTGTG

GAAAACACTAGCCGGCGACCCATGGATAAAAACACTGACTGCCAAGTACCCTGGGAATTTCA
CTGCCACGATAGAGGAGTGGCAAGCAGAGCATGATGCCATCATGAGGCACATCTTGGAGAGA
CCGGACCCTACCGACGTCTTCCAGAATAAGGCAAACGTGTGTTGGGCCAAGGCTTTAGTGCC
GGTGCTGAAGACCGCTGGCATAGACATGACCACTGAACAATGGAACACTGTGGATTATTTTG
AAACGGACAAAGCTCACTCAGCAGAGATAGTATTGAACCAACTATGCGTGAGGTTCTTTGGA
CTCGATCTGGACTCCGGTCTATTTTCTGCACCCACTGTTCCGTTATCCATTAGGAATAATCA
CTGGGATAACTCCCCGTCGCCTAACATGTACGGGCTGAATAAAGAAGTGGTCCGTCAGCTCT
CTCGCAGGTACCCACAACTGCCTCGGGCAGTTGCCACTGGAAGAGTCTATGACATGAACACT
GGTACACTGCGCAATTATGATCCGCGCATAAACCTAGTACCTGTAAACAGAAGACTGCCTCA
TGCTTTAGTCCTCCACCATAATGAACACCCACAGAGTGACTTTTCTTCATTCGTCAGCAAAT
TGAAGGGCAGAACTGTCCTGGTGGTCGGGGAAAAGTTGTCCGTCCCAGGCAAAATGGTTGAC
TGGTTGTCAGACCGGCCTGAGGCTACCTTCAGAGCTCGGCTGGATTTAGGCATCCCAGGTGA
TGTGCCCAAATATGACATAATATTTGTTAATGTGAGGACCCCATATAAATACCATCACTATC
AGCAGTGTGAAGACCATGCCATTAAGCTTAGCATGTTGACCAAGAAAGCTTGTCTGCATCTG
AATCCCGGCGGAACCTGTGTCAGCATAGGTTATGGTTACGCTGACAGGGCCAGCGAAAGCAT
CATTGGTGCTATAGCGCGGCAGTTCAAGTTTTCCCGGGTATGCAAACCGAAATCCTCACTTG
AAGAGACGGAAGTTCTGTTTGTATTCATTCGGTACGATCGCAAGGCCCGTACGCACAATTCT
TACAAGCTTTCATCAACCTTGACCAACATTTATACAGGTTCCAGACTCCACGAAGCCGGATG
TGCACCCTCATATCATGTGGTGCGAGGGGATATTGCCACGGCCACCGAAGGAGTGATTATAA
ATGCTGCTAACAGCAAAGGACAACCTGGCGGAGGGGTGTGCGGAGCGCTGTATAAGAAATTC
CCGGAAAGCTTCGATTTACAGCCGATCGAAGTAGGAAAAGCGCGACTGGTCAAAGGTGCAGC
TAAACATATCATTCATGCCGTAGGACCAAACTTCAACAAAGTTTCGGAGGTTGAAGGTGACA
AACAGTTGGCAGAGGCTTATGAGTCCATCGCTAAGATTGTCAACGATAACAATTACAAGTCA
GTAGCGATTCCACTGTTGTCCACCGGCATCTTTTCCGGGAACAAAGATCGACTAACCCAATC
ATTGAACCATTTGCTGACAGCTTTAGACACCACTGATGCAGATGTAGCCATATACTGCAGGG
ACAAGAAATGGGAAATGACTCTCAAGGAAGCAGTGGCTAGGAGAGAAGCAGTGGAGGAGATA
TGCATATCCGACGACTCTTCAGTGACAGAACCTGATGCAGAGCTGGTGAGGGTGCATCCGAA
GAGTTCTTTGGCTGGAAGGAAGGGCTACAGCACAAGCGATGGCAAAACTTTCTCATATTTGG
AAGGGACCAAGTTTCACCAGGCGGCCAAGGATATAGCAGAAATTAATGCCATGTGGCCCGTT
GCAACGGAGGCCAATGAGCAGGTATGCATGTATATCCTCGGAGAAGGCATGAGCAGTATTAG
GTCGAAATGCCCCGTCGAAGAGTCGGAAGCCTCCACACCACCTAGCACGCTGCCTTGCTTGT
GCATCCATGCCATGACTCCAGAAAGAGTACAGCGCCTAAAAGCCTCACGTCCAGAACAAATT
ACTGTGTGCTCATCCTTTCCATTGCCGAAGTATAGAATCACTGGTGTGCAGAAGATCCAATG
CTCCCAGCCTATATTGTTCTCACCGAAAGTGCCTGCGTATATTCATCCAAGGAAGTATCTCG
TGGAAACACCACCGGTAGACGAGACTCCGGAGCCATCGGCAGAGAACCAATCCACAGAGGGG
ACACCTGAACAACCACCACTTATAACCGAGGATGAGACCAGGACTAGAACGCCTGAGCCGAT
CATCATCGAAGAGGAAGAAGAGGATAGCATAAGTTTGCTGTCAGATGGCCCGACCCACCAGG
TGCTGCAAGTCGAGGCAGACATTCACGGGCCGCCCTCTGTATCTAGCTCATCCTGGTCCATT
CCTCATGCATCCGACTTTGATGTGGACAGTTTATCCATACTTGACACCCTGGAGGGAGCTAG
CGTGACCAGCGGGGCAACGTCAGCCGAGACTAACTCTTACTTCGCAAAGAGTATGGAGTTTC
TGGCGCGACCGGTGCCTGCGCCTCGAACAGTATTCAGGAACCCTCCACATCCCGCTCCGCGC
ACAAGAACACCGTCACTTGCACCCAGCAGGGCCTGCTCGAGAACCAGCCTAGTTTCCACCCC
GCCAGGCGTGAATAGGGTGATCACTAGAGAGGAGCTCGAGGCGCTTACCCCGTCACGCACTC
CTAGCAGGTCGGTCTCGAGAACCAGCCTGGTCTCCAACCCGCCAGGCGTAAATAGGGTGATT
ACAAGAGAGGAGTTTGAGGCGTTCGTAGCACAACAACAATGACGGTTTGATGCGGGTGCATA
CATCTTTTCCTCCGACACCGGTCAAGGGCATTTACAACAAAAATCAGTAAGGCAAACGGTGC
TATCCGAAGTGGTGTTGGAGAGGACCGAATTGGAGATTTCGTATGCCCCGCGCCTCGACCAA
GAAAAAGAAGAATTACTACGCAAGAAATTACAGTTAAATCCCACACCTGCTAACAGAAGCAG
ATACCAGTCCAGGAAGGTGGAGAACATGAAAGCCATAACAGCTAGACGTATTCTGCAAGGCC
TAGGGCATTATTTGAAGGCAGAAGGAAAAGTGGAGTGCTACCGAACCCTGCATCCTGTTCCT
TTGTATTCATCTAGTGTGAACCGTGCCTTTTCAAGCCCCAAGGTCGCAGTGGAAGCCTGTAA
CGCCATGTTGAAAGAGAACTTTCCGACTGTGGCTTCTTACTGTATTATTCCAGAGTACGATG

CCTATTTGGACATGGTTGACGGAGCTTCATGCTGCTTAGACACTGCCAGTTTTTGCCCTGCA
AAGCTGCGCAGCTTTCCAAAGAAACACTCCTATTTGGAACCCACAATACGATCGGCAGTGCC
TTCAGCGATCCAGAACACGCTCCAGAACGTCCTGGCAGCTGCCACAAAAAGAAATTGCAATG
TCACGCAAATGAGAGAATTGCCCGTATTGGATTCGGCGGCCTTTAATGTGGAATGCTTCAAG
AAATATGCGTGTAATAATGAATATTGGGAAACGTTTAAAGAAAACCCCATCAGGCTTACTGA
AGAAAACGTGGTAAATTACATTACCAAATTAAAAGGACCAAAAGCTGCTGCTCTTTTTGCGA
AGACACATAATTTGAATATGTTGCAGGACATACCAATGGACAGGTTTGTAATGGACTTAAAG
AGAGACGTGAAAGTGACTCCAGGAACAAAACATACTGAAGAACGGCCCAAGGTACAGGTGAT
CCAGGCTGCCGATCCGCTAGCAACAGCGTATCTGTGCGGAATCCACCGAGAGCTGGTTAGGA
GATTAAATGCGGTCCTGCTTCCGAACATTCATACACTGTTTGATATGTCGGCTGAAGACTTT
GACGCTATTATAGCCGAGCACTTCCAGCCTGGGGATTGTGTTCTGGAAACTGACATCGCGTC
GTTTGATAAAAGTGAGGACGACGCCATGGCTCTGACCGCGTTAATGATTCTGGAAGACTTAG
GTGTGGACGCAGAGCTGTTGACGCTGATTGAGGCGGCTTTCGGCGAAATTTCATCAATACAT
TTGCCCACTAAAACTAAATTTAAATTCGGAGCCATGATGAAATCTGGAATGTTCCTCACACT
GTTTGTGAACACAGTCATTAACATTGTAATCGCAAGCAGAGTGTTGAGAGAACGGCTAACCG
GATCACCATGTGCAGCATTCATTGGAGATGACAATATCGTGAAAGGAGTCAAATCGGACAAA
TTAATGGCAGACAGGTGCGCCACCTGGTTGAATATGGAAGTCAAGATTATAGATGCTGTGGT
GGGCGAGAAAGCGCCTTATTTCTGTGGAGGGTTTATTTTGTGTGACTCCGTGACCGGCACAG
CGTGCCGTGTGGCAGACCCCCTAAAAAGGCTGTTTAAGCTTGGCAAACCTCTGGCAGCAGAC
GATGAACATGATGATGACAGGAGAAGGGCATTGCATGAAGAGTCAACACGCTGGAACCGAGT
GGGTATTCTTTCAGAGCTGTGCAAGGCAGTAGAATCAAGGTATGAAACCGTAGGAACTTCCA
TCATAGTTATGGCCATGACTACTCTAGCTAGCAGTGTTAAATCATTCAGCTACCTGAGAGGG
GCCCCTATAACTCTCTACGGCTAACCTGAATGGACTACGACATAGTCTAGTCCGCCAAGTCT
AGCATATGGGCGCGCCCTCAGCATCGATTGAATTGGCCACCATGGTGAGCAAGGGCGAGGAG
GATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAA
CGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCG
CCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAG
TTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCT
GTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGA
CCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGC
ACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTC
CGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGA
AGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAG
CTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACAC
CATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGT
ACAAGTAGGAATTGGCAAGCTGCTTACATAGAACTCGCGGCGATTGGCATGCCGCCTTAAAA
TTTTTATTTTATTTTTCTTTTCTTTTCCGAATCGGATTTTGTTTTTAATATTTCAAAAAAAA
AAAAAAAAAAAAAAAAAACGCGTCGAGGGGAATTAATTCTTGAAGACGAAAGGGCCAGGTGG
CACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATA
TGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGT
ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGT
TTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAG
TGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAA
CGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGA
CGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACT
CACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCC
ATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGA
GCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGG
AGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACA
ACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGA
CTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGT
TTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGG

CCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGA
TGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAG
ACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATC
TAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCA
CTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCG
TAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAA
GAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGT
CCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACC
TCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGG
TTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTG
CACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATT
GAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTC
GGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGT
CGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCC
TATGGAAAAACGCCAGCAACGCGAGCTCTAATACGACTCACTATAG (SEQ ID NO: 2)

[0051] In other instances, the self-amplifying replicon RNA is derived from a Hepatitis C virus (HCV). HCV are part of the Group IV Flaviviridae family of viruses, possessing monopartite, linear, single-stranded RNA genomes of positive polarity, 9.6 to 12.3 kilobase in length. The 5'-termini of flaviviruses carry a methylated nucleotide cap, while other members of this family are uncapped and encode an internal ribosome entry site.

[0052] In other instances, the alphavirus is a SF virus. The SF virus is a viral pathogen typically carried by mosquitos that causes encephalitis. The Semliki Forest virus is a positive-stranded RNA virus with a genome of approximately 13,000 base pairs which encodes nine proteins. The 5' two thirds of the genome encode four non-structural proteins concerned with RNA synthesis and the structural proteins are encoded in the 3' third. Of the structural proteins, the C proteins makes up the icosahedral capsid which is enveloped by a lipid bilayer, derived from the host cell. The outermost surface of the virus is almost entirely covered by heterodimers of glycoproteins E1 and E2, arranged in inter-connective trimers, which form an outer shell. Trimers are anchored in the membrane by an E2 cytoplasmic domain that associates with the nucleocapsid.

[0053] In other instances, the alphavirus is a SIN virus. The virus is transmitted by mosquitoes. SIN virus causes Sindbis fever in humans and the symptoms include arthralgia, rash and malaise. Sindbis viruses are enveloped particles with an icosahedral capsid. Its genome is a single stranded RNA approximately 11.7kb long. It has a 5' cap and 3' polyadenylated tail therefore serves directly as messenger RNA (mRNA) in a host cell. The genome encodes four non-structural proteins at the 5' end and the capsid and two envelope proteins at the 3' end. This is characteristic of all Togaviruses. Replication is cytoplasmic and rapid. The genomic RNA is partially translated at the 5' end to produce the non-structural proteins which are then involved in genome replication and the production of new genomic RNA and a shorter sub-genomic RNA strand. This sub-genomic strand is translated into the structural proteins. The viruses assemble at the host cell surfaces and acquire their envelope through budding. A non-coding RNA element has been found to be essential for Sindbis virus genome replication.

[0054] The viral replicon RNA, is capable of stimulating immune response via toll like receptors (TLRs). A subset of TLRs, TLR3, TLR7/8, and TLR9, is involved in antiviral responses by triggering the production of antiviral cytokines such as type I interferons (IFNs). TLR3 responds to double stranded RNA, a replication intermediary for many viruses. TLR7/8 recognize viral single-stranded RNAs, whereas TLR9 recognizes unmethylated CpG motifs within viral DNA. TLRs involved in virus recognition are expressed on endosomal membranes and can be separated according to their requirement for the adaptor protein MyD88: TLR3 activity is MyD88-independent while TLRs7/8/9 depend on MyD88. The activation of TLR3 leads to the production of Type I Interferon (IFN). Type-I interferon signaling through ISGF3 (STAT1/STAT2/IRF9) complex is required for sustained Rip3 activation and necroptosis.

[0055] In some instances, the LNP-replicon RNA are capable of stimulating TLR3 signaling in tumor cells, which leads to necrotic cell death of tumor cells. In some instances, the LNP-replicon RNA can enhance the immunogenic cell death (ICD) induced by the LNPs. In some instances, TT3-LNP-Replicon RNA can exert tumor inhibition by synergistically induce ICD in tumor cells and trigger an anti-tumor immune response (e.g., recruiting of immune cells such as granulocytes, monocytes, macrophages, myeloid derived suppressive cells, dendritic cells, T cells, and NK cells). In some instances, the synthetic oncolytic virus comprises TT3-LNP-mtReplicon RNA.

[0056] In some instances, the replicon RNA comprises a coding sequence for a detectable molecule in the subgenomic region. In some instances, the detectable molecule is a nucleic acid or a polypeptide. In some instances, the polypeptide is a fluorescent protein. Fluorescent proteins are known in the art, and are a subclass of fluorophores, which are fluorescent

chemical compounds with the ability to re-emit light upon excitation. The fluorophore will absorb excitation light energy of a first specific wavelength, and then will re-emit light energy at a second, longer specific wavelength. Each type of fluorophore responds to and emits differing wavelengths of light, depending on the nature of its chemical structure and environment. In some instances, the fluorescent protein includes, but is not limited to, wt-GFP, green fluorescent protein (e.g., EGFP, Emerald, Superfolder GFP, Azami Green, mWasabi, TagGFP, TurboGFP, AcGFP, ZsGreen, T-Sapphire, etc.), blue fluorescent protein, (*e.g.,* EBFP, EBFP2, Azurite, mTagBFP, etc.), cyan fluorescent protein (*e.g.,* ECFP, mECFP, Cerulean, mTurquoise, CyPet, AmCyan1, Midori-Ishi Cyan, TagCFP, mTFP1 (Teal), etc.), yellow fluorescent protein (*e.g.*, EYFP, Topaz, Venus, mCitrine, YPet, TagYFP, PhiYFP, ZsYellow1, mBanana, etc.), orange fluorescent protein (*e.g.*, Kusabira Orange, Kusabira Orange2, mOrange, mOrange2, dTomato, dTomato-Tandem, TagRFP, TagRFP-T, DsRed, DsRed2, DsRed-Express (T1), DsRed-Monomer, mTangerine, etc.), or red fluorescent protein (*e.g.*, mRuby, mApple, mStrawberry, AsRed2, mRFP1, JRed, mCherry, HcRed1, mRaspberry, dKeima-Tandem, HcRed-Tandem, mPlum, AQ143, etc.).

[0057] In some aspects, as described herein, the self-amplifying replicon RNA comprises a coding sequence for expression of IL-12 in the subgenomic region. An exemplary coding sequence for IL-12 is set forth in SEQ ID NO: 3:

ATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCACGATGTGC
CATGTGGGAGCTGGAGAAAGACGTTTATGTTGTAGAGGTGGACTGGACTCCCGATGCCCCTG
GAGAAACAGTGAACCTCACCTGTGACACGCCTGAAGAAGATGACATCACCTGGACCTCAGAC
CAGAGACATGGAGTCATAGGCTCTGGAAAGACCCTGACCATCACTGTCAAAGAGTTTCTAGA
TGCTGGCCAGTACACCTGCCACAAAGGAGGCGAGACTCTGAGCCACTCACATCTGCTGCTCC
ACAAGAAGGAAAATGGAATTTGGTCCACTGAAATTTTAAAAAATTTCAAAAACAAGACTTTC
CTGAAGTGTGAAGCACCAAATTACTCCGGACGGTTCACGTGCTCATGGCTGGTGCAAAGAAA
CATGGACTTGAAGTTCAACATCAAGAGCAGTAGCAGTTCCCCTGACTCTCGGGCAGTGACAT
GTGGAATGGCGTCTCTGTCTGCAGAGAAGGTCACACTGGACCAAAGGGACTATGAGAAGTAT
TCAGTGTCCTGCCAGGAGGATGTCACCTGCCCAACTGCCGAGGAGACCCTGCCCATTGAACT
GGCGTTGGAAGCACGGCAGCAGAATAAATATGAGAACTACAGCACCAGCTTCTTCATCAGGG
ACATCATCAAACCAGACCCGCCCAAGAACTTGCAGATGAAGCCTTTGAAGAACTCACAGGTG
GAGGTCAGCTGGGAGTACCCTGACTCCTGGAGCACTCCCCATTCCTACTTCTCCCTCAAGTT
CTTTGTTCGAATCCAGCGCAAGAAAGAAAAGATGAAGGAGACAGAGGAGGGGTGTAACCAGA
AAGGTGCGTTCCTCGTAGAGAAGACATCTACCGAAGTCCAATGCAAAGGCGGGAATGTCTGC
GTGCAAGCTCAGGATCGCTATTACAATTCCTCATGCAGCAAGTGGGCATGTGTTCCCTGCAG
GGTCCGATCCGGAGGTTCCGGTGGTGGATCCGGAGGTGGCTCCGGCGGCGGATCCAGGGTCA
TTCCAGTCTCTGGACCTGCCAGGTGTCTTAGCCAGTCCCGAAACCTGCTGAAGACCACAGAT
GACATGGTGAAGACGGCCAGAGAAAAACTGAAACATTATTCCTGCACTGCTGAAGACATCGA
TCATGAAGACATCACACGGGACCAAACCAGCACATTGAAGACCTGTTTACCACTGGAACTAC
ACAAGAACGAGAGTTGCCTGGCTACTAGAGAGACTTCTTCCACAACAAGAGGGAGCTGCCTG
CCCCCACAGAAGACGTCTTTGATGATGACCCTGTGCCTTGGTAGCATCTATGAGGACTTGAA
GATGTACCAGACAGAGTTCCAGGCCATCAACGCAGCACTTCAGAATCACAACCATCAGCAGA
TCATTCTAGACAAGGGCATGCTGGTGGCCATCGATGAGCTGATGCAGTCTCTGAATCATAAT
GGCGAGACTCTGCGCCAGAAACCTCCTGTGGGAGAAGCAGACCCTTACAGAGTGAAAATGAA
GCTCTGCATCCTGCTTCACGCCTTCAGCACCCGCGTCGTGACCATCAACAGGGTGATGGGCT
ATCTGAGCTCCGCCTGA (SEQ ID NO: 3)

[0058] Structurally, IL-12 belongs to type I cytokines and has a four α-helical bundle structure. IL-12 acts in a form of a heterodimeric protein (IL-12-p70; IL-12-p30/p40) consisting of two covalently linked p30 and p40 subunits. Contrary to the heterodimeric form, IL-12-p40/p40 homodimer acts mostly as a competitive suppressant of IL-12-p70 actions. IL-12 is a pleiotropic cytokine, the actions of which create an interconnection between the innate and adaptive immunity. IL-12 was first described as a factor secreted from PMA-induced EBV-transformed B-cell lines. Based on its actions, IL-12 was initially designated as "cytotoxic lymphocyte maturation factor" and "natural killer cell stimulatory factor". Due to bridging the innate and adaptive immunity and potently stimulating the production of IFN-γ, a cytokine coordinating natural mechanisms of anticancer defense, IL-12 seemed an ideal candidate for tumor immunotherapy in humans. However, severe side effects associated with systemic administration of IL-12 in clinical investigations and the very narrow therapeutic index of this cytokine markedly tempered enthusiasm for the use of this cytokine in cancer patients.

[0059] Following the discovery of IL-12, three other members (IL-23, IL-27, and IL-35) have been added to the IL-12 family and shown to play critical roles in Th1 cell functions. IL-12 is a ligand of a receptor composed of two amino acid chains, IL-12R-β1 and IL-12R-β2. IL-12 receptor is expressed in a constitutive (e.g., IL-12R-β1 in B cells) or inducible (IL-12R-β2) manner in a variety of immune cells, including NK cells, T, and B lymphocytes. Ligand-bound IL-12R-β2 becomes phosphorylated on tyrosines, which provides harboring sites for two kinases, JAK2 and TYK2. Among the STAT family of transcription factors, STAT4 is considered to be the most specific mediator of cellular responses elicited by IL-12. The main elements of IL-12 actions are as follows: increasing production of IFN-γ, which is the most potent mediator of IL-12 actions, from NK and T cells; stimulation of growth and cytotoxicity of activated NK cells, CD8+ and CD4+ T cells, shifting differentiation of CD4+ Th0 cells toward the Th1 phenotype; enhancement of antibody-dependent cellular cytotoxicity (ADCC) against tumor cells; and the induction of IgG and suppression of IgE production from B cells. The main source of IL-12 in humans is the activated antigen-presenting cells, such as dendritic cells, especially of the CD1c+ phenotype, as well as the hematopoietic phagocytes (monocytes, macrophages, and also neutrophils), but IL-12 can also be produced by other cell types. While IL-12 acts on a variety of immune cells, the overall physiological role for IL-12 seems to be orchestrating the Th1-type immune response against certain pathogens.

[0060] The present disclosure, in some aspect, describes local delivery of IL-12 by a LNP encapsulated viral replicon RNA, which encodes an IL-12 molecule in its subgenomic region. In addition to the synergistic tumor cell immunogenic cell death (ICD) induced by the LNP-replicon RNA, in some instances, the expression of IL12 in the tumor microenvironment can lead to further immunostimulation and results in an enhancement of anti-tumor immune response. In some instances, the combination of LNP-replicon RNA-IL-12 in the tumor microenvironment is capable of induce a systemic anti-tumor immune response. In other instances, the combination of LNP-replicon RNA-IL-12 in the tumor microenvironment is capable of eradicate the tumor cells and prevent the recurrence of the tumor. In some instances, the synthetic oncolytic virus comprises TT3-LNP-mtReplicon RNA-IL12.

[0061] In some instances, the replicon RNA-IL12 further comprises a coding sequence for serum albumin. The half-life of peptides and proteins (e.g., cytokines) in a biological environment (e.g., serum, tumor microenvironment) is affected by several factors, including size, charge, proteolytic sensitivity, nature of their biology, turnover rate of proteins they bind, and other factors. In some cases, the half-life of proteins in biological environment can be roughly correlated with their size. Peptides and proteins smaller than approximately 70 kDa (e.g., cytokines) can be eliminated via kidney filtration, so they generally possess very short half-lives. Larger proteins, however, may persist for several days. Three types of proteins, IgGs, serum albumin, and transferrin, persist for much longer than would be predicted just by their size. In some instances, the serum albumin is human serum albumin. In some instances, the serum albumin is mouse serum albumin (MSA). An exemplary coding sequence for replicon RNA-IL12-MSA is set forth in SEQ ID NO: 4:

ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAAATGGAGAAAGTTCACGTT
GACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGGAGCTTCCCGCAGTTTGAGGT
AGAAGCCAAGCAGGTCACTGATAATGACCATGCTAATGCCAGAGCGTTTTCGCATCTGGCTT
CAAAACTGATCGAAACGGAGGTGGACCCATCCGACACGATCCTTGACATTGGAAGTGCGCCC
GCCCGCAGAATGTATTCTAAGCACAAGTATCATTGTATCTGTCCGATGAGATGTGCGGAAGA
TCCGGACAGATTGTATAAGTATGCAACTAAGCTGAAGAAAAACTGTAAGGAAATAACTGATA
AGGAATTGGACAAGAAAATGAAGGAGCTGGCCGCCGTCATGAGCGACCCTGACCTGGAAACT
GAGACTATGTGCCTCCACGACGACGAGTCGTGTCGCTACGAAGGGCAAGTCGCTGTTTACCA
GGATGTATACGCGGTTGACGGACCGACAAGTCTCTATCACCAAGCCAATAAGGGAGTTAGAG
TCGCCTACTGGATAGGCTTTGACACCACCCCTTTTATGTTTAAGAACTTGGCTGGAGCATAT
CCATCATACTCTACCAACTGGGCCGACGAAACCGTGTTAACGGCTCGTAACATAGGCCTATG
CAGCTCTGACGTTATGGAGCGGTCACGTAGAGGGATGTCCATTCTTAGAAAGAAGTATTTGA
AACCATCCAACAATGTTCTATTCTCTGTTGGCTCGACCATCTACCACGAGAAGAGGGACTTA
CTGAGGAGCTGGCACCTGCCGTCTGTATTTCACTTACGTGGCAAGCAAAATTACACATGTCG
GTGTGAGACTATAGTTAGTTGCGACGGGTACGTCGTTAAAAGAATAGCTATCAGTCCAGGCC
TGTATGGGAAGCCTTCAGGCTATGCTGCTACGATGCACCGCGAGGGATTCTTGTGCTGCAAA
GTGACAGACACATTGAACGGGGAGAGGGTCTCTTTTCCCGTGTGCACGTATGTGCCAGCTAC
ATTGTGTGACCAAATGACTGGCATACTGGCAACAGATGTCAGTGCGGACGACGCGCAAAAAC
TGCTGGTTGGGCTCAACCAGCGTATAGTCGTCAACGGTCGCACCCAGAGAAACACCAATACC
ATGAAAAATTACCTTTTGCCCGTAGTGGCCCAGGCATTTGCTAGGTGGGCAAAGGAATATAA
GGAAGATCAAGAAGATGAAAGGCCACTAGGACTACGAGATAGACAGTTAGTCATGGGGTGTT
GTTGGGCTTTTAGAAGGCACAAGATAACATCTATTTATAAGCGCCCGGATACCCAAACCATC
ATCAAAGTGAACAGCGATTTCCACTCATTCGTGCTGCCCAGGATAGGCAGTAACACATTGGA
GATCGGGCTGAGAACAAGAATCAGGAAAATGTTAGAGGAGCACAAGGAGCCGTCACCTCTCA
TTACCGCCGAGGACGTACAAGAAGCTAAGTGCGCAGCCGATGAGGCTAAGGAGGTGCGTGAA
GCCGAGGAGTTGCGCGCAGCTCTACCACCTTTGGCAGCTGATGTTGAGGAGCCCACTCTGGA
AGCCGATGTCGACTTGATGTTACAAGAGGCTGGGGCCGGCTCAGTGGAGACACCTCGTGGCT
TGATAAAGGTTACCAGCTACGATGGCGAGGACAAGATCGGCTCTTACGCTGTGCTTTCTCCG

CAGGCTGTACTCAAGAGTGAAAAATTATCTTGCATCCACCCTCTCGCTGAACAAGTCATAGT
GATAACACACTCTGGCCGAAAAGGGCGTTATGCCGTGGAACCATACCATGGTAAAGTAGTGG
TGCCAGAGGGACATGCAATACCCGTCCAGGACTTTCAAGCTCTGAGTGAAAGTGCCACCATT
GTGTACAACGAACGTGAGTTCGTAAACAGGTACCTGCACCATATTGCCACACATGGAGGAGC
GCTGAACACTGATGAAGAATATTACAAAACTGTCAAGCCCAGCGAGCACGACGGCGAATACC
TGTACGACATCGACAGGAAACAGTGCGTCAAGAAAGAACTAGTCACTGGGCTAGGGCTCACA
GGCGAGCTGGTGGATCCTCCCTTCCATGAATTCGCCTACGAGAGTCTGAGAACACGACCAGC
CGCTCCTTACCAAGTACCAACCATAGGGGTGTATGGCGTGCCAGGATCAGGCAAGTCTGGCA
TCATTAAAAGCGCAGTCACCAAAAAAGATCTAGTGGTGAGCGCCAAGAAAGAAAACTGTGCA
GAAATTATAAGGGACGTCAAGAAAATGAAAGGGCTGGACGTCAATGCCAGAACTGTGGACTC
AGTGCTCTTGAATGGATGCAAACACCCCGTAGAGACCCTGTATATTGACGAAGCTTTTGCTT
GTCATGCAGGTACTCTCAGAGCGCTCATAGCCATTATAAGACCTAAAAAGGCAGTGCTCTGC
GGGGATCCCAAACAGTGCGGTTTTTTTAACATGATGTGCCTGAAAGTGCATTTTAACCACGA
GATTTGCACACAAGTCTTCCACAAAAGCATCTCTCGCCGTTGCACTAAATCTGTGACTTCGG
TCGTCTCAACCTTGTTTTACGACAAAAAAATGAGAACGACGAATCCGAAAGAGACTAAGATT
GTGATTGACACTACCGGCAGTACCAAACCTAAGCAGGACGATCTCATTCTCACTTGTTTCAG
AGGGTGGGTGAAGCAGTTGCAAATAGATTACAAAGGCAACGAAATAATGACGGCAGCTGCCT
CTCAAGGGCTGACCCGTAAAGGTGTGTATGCCGTTCGGTACAAGGTGAATGAAAATCCTCTG
TACGCACCCACCTCAGAACATGTGAACGTCCTACTGACCCGCACGGAGGACCGCATCGTGTG
GAAAACACTAGCCGGCGACCCATGGATAAAAACACTGACTGCCAAGTACCCTGGGAATTTCA
CTGCCACGATAGAGGAGTGGCAAGCAGAGCATGATGCCATCATGAGGCACATCTTGGAGAGA
CCGGACCCTACCGACGTCTTCCAGAATAAGGCAAACGTGTGTTGGGCCAAGGCTTTAGTGCC
GGTGCTGAAGACCGCTGGCATAGACATGACCACTGAACAATGGAACACTGTGGATTATTTTG
AAACGGACAAAGCTCACTCAGCAGAGATAGTATTGAACCAACTATGCGTGAGGTTCTTTGGA
CTCGATCTGGACTCCGGTCTATTTTCTGCACCCACTGTTCCGTTATCCATTAGGAATAATCA
CTGGGATAACTCCCCGTCGCCTAACATGTACGGGCTGAATAAAGAAGTGGTCCGTCAGCTCT
CTCGCAGGTACCCACAACTGCCTCGGGCAGTTGCCACTGGAAGAGTCTATGACATGAACACT
GGTACACTGCGCAATTATGATCCGCGCATAAACCTAGTACCTGTAAACAGAAGACTGCCTCA
TGCTTTAGTCCTCCACCATAATGAACACCCACAGAGTGACTTTTCTTCATTCGTCAGCAAAT
TGAAGGGCAGAACTGTCCTGGTGGTCGGGGAAAAGTTGTCCGTCCCAGGCAAAATGGTTGAC
TGGTTGTCAGACCGGCCTGAGGCTACCTTCAGAGCTCGGCTGGATTTAGGCATCCCAGGTGA
TGTGCCCAAATATGACATAATATTTGTTAATGTGAGGACCCCATATAAATACCATCACTATC
AGCAGTGTGAAGACCATGCCATTAAGCTTAGCATGTTGACCAAGAAAGCTTGTCTGCATCTG
AATCCCGGCGGAACCTGTGTCAGCATAGGTTATGGTTACGCTGACAGGGCCAGCGAAAGCAT
CATTGGTGCTATAGCGCGGCAGTTCAAGTTTTCCCGGGTATGCAAACCGAAATCCTCACTTG
AAGAGACGGAAGTTCTGTTTGTATTCATTCGGTACGATCGCAAGGCCCGTACGCACAATTCT
TACAAGCTTTCATCAACCTTGACCAACATTTATACAGGTTCCAGACTCCACGAAGCCGGATG
TGCACCCTCATATCATGTGGTGCGAGGGGATATTGCCACGGCCACCGAAGGAGTGATTATAA
ATGCTGCTAACAGCAAAGGACAACCTGGCGGAGGGGTGTGCGGAGCGCTGTATAAGAAATTC
CCGGAAAGCTTCGATTTACAGCCGATCGAAGTAGGAAAAGCGCGACTGGTCAAAGGTGCAGC
TAAACATATCATTCATGCCGTAGGACCAAACTTCAACAAAGTTTCGGAGGTTGAAGGTGACA
AACAGTTGGCAGAGGCTTATGAGTCCATCGCTAAGATTGTCAACGATAACAATTACAAGTCA
GTAGCGATTCCACTGTTGTCCACCGGCATCTTTTCCGGGAACAAAGATCGACTAACCCAATC
ATTGAACCATTTGCTGACAGCTTTAGACACCACTGATGCAGATGTAGCCATATACTGCAGGG
ACAAGAAATGGGAAATGACTCTCAAGGAAGCAGTGGCTAGGAGAGAAGCAGTGGAGGAGATA
TGCATATCCGACGACTCTTCAGTGACAGAACCTGATGCAGAGCTGGTGAGGGTGCATCCGAA
GAGTTCTTTGGCTGGAAGGAAGGGCTACAGCACAAGCGATGGCAAAACTTTCTCATATTTGG
AAGGGACCAAGTTTCACCAGGCGGCCAAGGATATAGCAGAAATTAATGCCATGTGGCCCGTT
GCAACGGAGGCCAATGAGCAGGTATGCATGTATATCCTCGGAGAAGGCATGAGCAGTATTAG
GTCGAAATGCCCCGTCGAAGAGTCGGAAGCCTCCACACCACCTAGCACGCTGCCTTGCTTGT
GCATCCATGCCATGACTCCAGAAAGAGTACAGCGCCTAAAAGCCTCACGTCCAGAACAAATT
ACTGTGTGCTCATCCTTTCCATTGCCGAAGTATAGAATCACTGGTGTGCAGAAGATCCAATG

CTCCCAGCCTATATTGTTCTCACCGAAAGTGCCTGCGTATATTCATCCAAGGAAGTATCTCG
TGGAAACACCACCGGTAGACGAGACTCCGGAGCCATCGGCAGAGAACCAATCCACAGAGGGG
ACACCTGAACAACCACCACTTATAACCGAGGATGAGACCAGGACTAGAACGCCTGAGCCGAT
CATCATCGAAGAGGAAGAAGAGGATAGCATAAGTTTGCTGTCAGATGGCCCGACCCACCAGG
TGCTGCAAGTCGAGGCAGACATTCACGGGCCGCCCTCTGTATCTAGCTCATCCTGGTCCATT
CCTCATGCATCCGACTTTGATGTGGACAGTTTATCCATACTTGACACCCTGGAGGGAGCTAG
CGTGACCAGCGGGGCAACGTCAGCCGAGACTAACTCTTACTTCGCAAAGAGTATGGAGTTTC
TGGCGCGACCGGTGCCTGCGCCTCGAACAGTATTCAGGAACCCTCCACATCCCGCTCCGCGC
ACAAGAACACCGTCACTTGCACCCAGCAGGGCCTGCTCGAGAACCAGCCTAGTTTCCACCCC
GCCAGGCGTGAATAGGGTGATCACTAGAGAGGAGCTCGAGGCGCTTACCCCGTCACGCACTC
CTAGCAGGTCGGTCTCGAGAACCAGCCTGGTCTCCAACCCGCCAGGCGTAAATAGGGTGATT
ACAAGAGAGGAGTTTGAGGCGTTCGTAGCACAACAACAATGACGGTTTGATGCGGGTGCATA
CATCTTTTCCTCCGACACCGGTCAAGGGCATTTACAACAAAAATCAGTAAGGCAAACGGTGC
TATCCGAAGTGGTGTTGGAGAGGACCGAATTGGAGATTTCGTATGCCCCGCGCCTCGACCAA
GAAAAAGAAGAATTACTACGCAAGAAATTACAGTTAAATCCCACACCTGCTAACAGAAGCAG
ATACCAGTCCAGGAAGGTGGAGAACATGAAAGCCATAACAGCTAGACGTATTCTGCAAGGCC
TAGGGCATTATTTGAAGGCAGAAGGAAAAGTGGAGTGCTACCGAACCCTGCATCCTGTTCCT
TTGTATTCATCTAGTGTGAACCGTGCCTTTTCAAGCCCCAAGGTCGCAGTGGAAGCCTGTAA
CGCCATGTTGAAAGAGAACTTTCCGACTGTGGCTTCTTACTGTATTATTCCAGAGTACGATG
CCTATTTGGACATGGTTGACGGAGCTTCATGCTGCTTAGACACTGCCAGTTTTTGCCCTGCA
AAGCTGCGCAGCTTTCCAAAGAAACACTCCTATTTGGAACCCACAATACGATCGGCAGTGCC
TTCAGCGATCCAGAACACGCTCCAGAACGTCCTGGCAGCTGCCACAAAAAGAAATTGCAATG
TCACGCAAATGAGAGAATTGCCCGTATTGGATTCGGCGGCCTTTAATGTGGAATGCTTCAAG
AAATATGCGTGTAATAATGAATATTGGGAAACGTTTAAAGAAAACCCCATCAGGCTTACTGA
AGAAAACGTGGTAAATTACATTACCAAATTAAAAGGACCAAAAGCTGCTGCTCTTTTTGCGA
AGACACATAATTTGAATATGTTGCAGGACATACCAATGGACAGGTTTGTAATGGACTTAAAG
AGAGACGTGAAAGTGACTCCAGGAACAAAACATACTGAAGAACGGCCCAAGGTACAGGTGAT
CCAGGCTGCCGATCCGCTAGCAACAGCGTATCTGTGCGGAATCCACCGAGAGCTGGTTAGGA
GATTAAATGCGGTCCTGCTTCCGAACATTCATACACTGTTTGATATGTCGGCTGAAGACTTT
GACGCTATTATAGCCGAGCACTTCCAGCCTGGGGATTGTGTTCTGGAAACTGACATCGCGTC
GTTTGATAAAAGTGAGGACGACGCCATGGCTCTGACCGCGTTAATGATTCTGGAAGACTTAG
GTGTGGACGCAGAGCTGTTGACGCTGATTGAGGCGGCTTTCGGCGAAATTTCATCAATACAT
TTGCCCACTAAAACTAAATTTAAATTCGGAGCCATGATGAAATCTGGAATGTTCCTCACACT
GTTTGTGAACACAGTCATTAACATTGTAATCGCAAGCAGAGTGTTGAGAGAACGGCTAACCG
GATCACCATGTGCAGCATTCATTGGAGATGACAATATCGTGAAAGGAGTCAAATCGGACAAA
TTAATGGCAGACAGGTGCGCCACCTGGTTGAATATGGAAGTCAAGATTATAGATGCTGTGGT
GGGCGAGAAAGCGCCTTATTTCTGTGGAGGGTTTATTTTGTGTGACTCCGTGACCGGCACAG
CGTGCCGTGTGGCAGACCCCCTAAAAAGGCTGTTTAAGCTTGGCAAACCTCTGGCAGCAGAC
GATGAACATGATGATGACAGGAGAAGGGCATTGCATGAAGAGTCAACACGCTGGAACCGAGT
GGGTATTCTTTCAGAGCTGTGCAAGGCAGTAGAATCAAGGTATGAAACCGTAGGAACTTCCA
TCATAGTTATGGCCATGACTACTCTAGCTAGCAGTGTTAAATCATTCAGCTACCTGAGAGGG
GCCCCTATAACTCTCTACGGCTAACCTGAATGGACTACGACATAGTCTAGTCCGCCAAGTCT
AGCATATGGGCGCGCCCTCAGCATCGATTTGAATTGGCCACCATGAGGGTCCCCGCTCAGCT
CCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCACGATGTGCCATGTGGGAGCTGGAGAAAG
ACGTTTATGTTGTAGAGGTGGACTGGACTCCCGATGCCCCTGGAGAAACAGTGAACCTCACC
TGTGACACGCCTGAAGAAGATGACATCACCTGGACCTCAGACCAGAGACATGGAGTCATAGG
CTCTGGAAAGACCCTGACCATCACTGTCAAAGAGTTTCTAGATGCTGGCCAGTACACCTGCC
ACAAAGGAGGCGAGACTCTGAGCCACTCACATCTGCTGCTCCACAAGAAGGAAAATGGAATT
TGGTCCACTGAAATTTTAAAAAATTTCAAAAACAAGACTTTCCTGAAGTGTGAAGCACCAAA
TTACTCCGGACGGTTCACGTGCTCATGGCTGGTGCAAAGAAACATGGACTTGAAGTTCAACA
TCAAGAGCAGTAGCAGTTCCCCTGACTCTCGGGCAGTGACATGTGGAATGGCGTCTCTGTCT
GCAGAGAAGGTCACACTGGACCAAAGGGACTATGAGAAGTATTCAGTGTCCTGCCAGGAGGA

TGTCACCTGCCCAACTGCCGAGGAGACCCTGCCCATTGAACTGGCGTTGGAAGCACGGCAGC
AGAATAAATATGAGAACTACAGCACCAGCTTCTTCATCAGGGACATCATCAAACCAGACCCG
CCCAAGAACTTGCAGATGAAGCCTTTGAAGAACTCACAGGTGGAGGTCAGCTGGGAGTACCC
TGACTCCTGGAGCACTCCCCATTCCTACTTCTCCCTCAAGTTCTTTGTTCGAATCCAGCGCA
AGAAAGAAAAGATGAAGGAGACAGAGGAGGGGTGTAACCAGAAAGGTGCGTTCCTCGTAGAG
AAGACATCTACCGAAGTCCAATGCAAAGGCGGGAATGTCTGCGTGCAAGCTCAGGATCGCTA
TTACAATTCCTCATGCAGCAAGTGGGCATGTGTTCCCTGCAGGGTCCGATCCGGAGGTTCCG
GTGGTGGATCCGGAGGTGGCTCCGGCGGCGGATCCAGGGTCATTCCAGTCTCTGGACCTGCC
AGGTGTCTTAGCCAGTCCCGAAACCTGCTGAAGACCACAGATGACATGGTGAAGACGGCCAG
AGAAAAACTGAAACATTATTCCTGCACTGCTGAAGACATCGATCATGAAGACATCACACGGG
ACCAAACCAGCACATTGAAGACCTGTTTACCACTGGAACTACACAAGAACGAGAGTTGCCTG
GCTACTAGAGAGACTTCTTCCACAACAAGAGGGAGCTGCCTGCCCCCACAGAAGACGTCTTT
GATGATGACCCTGTGCCTTGGTAGCATCTATGAGGACTTGAAGATGTACCAGACAGAGTTCC
AGGCCATCAACGCAGCACTTCAGAATCACAACCATCAGCAGATCATTCTAGACAAGGGCATG
CTGGTGGCCATCGATGAGCTGATGCAGTCTCTGAATCATAATGGCGAGACTCTGCGCCAGAA
ACCTCCTGTGGGAGAAGCAGACCCTTACAGAGTGAAAATGAAGCTCTGCATCCTGCTTCACG
CCTTCAGCACCCGCGTCGTGACCATCAACAGGGTGATGGGCTATCTGAGCTCCGCCGGTTCC
GGTGGCGGATCCGAAGCACACAAGAGTGAGATCGCCCATCGGTATAATGATTTGGGAGAACA
ACATTTCAAAGGCCTAGTCCTGATTGCCTTTTCCCAGTATCTCCAGAAATGCTCATACGATG
AGCATGCCAAATTAGTGCAGGAAGTAACAGACTTTGCAAAGACGTGTGTTGCCGATGAGTCT
GCCGCCAACTGTGACAAATCCCTTCACACTCTTTTTGGAGATAAGTTGTGTGCCATTCCAAA
CCTCCGTGAAAACTATGGTGAACTGGCTGACTGCTGTACAAAACAAGAGCCCGAAAGAAACG
AATGTTTCCTGCAACACAAAGATGACAACCCCAGCCTGCCACCATTTGAAAGGCCAGAGGCT
GAGGCCATGTGCACCTCCTTTAAGGAAAACCCAACCACCTTTATGGGACACTATTTGCATGA
AGTTGCCAGAAGACATCCTTATTTCTATGCCCCAGAACTTCTTTACTATGCTGAGCAGTACA
ATGAGATTCTGACCCAGTGTTGTGCAGAGGCTGACAAGGAAAGCTGCCTGACCCCGAAGCTT
GATGGTGTGAAGGAGAAAGCATTGGTCTCATCTGTCCGTCAGAGAATGAAGTGCTCCAGTAT
GCAGAAGTTTGGAGAGAGAGCTTTTAAAGCATGGGCAGTAGCTCGTCTGAGCCAGACATTCC
CCAATGCTGACTTTGCAGAAATCACCAAATTGGCAACAGACCTGACCAAAGTCAACAAGGAG
TGCTGCCATGGTGACCTGCTGGAATGCGCAGATGACAGGGCGGAACTTGCCAAGTACATGTG
TGAAAACCAGGCGACTATCTCCAGCAAACTGCAGACTTGCTGCGATAAACCACTGTTGAAGA
AAGCCCACTGTCTTAGTGAGGTGGAGCATGACACCATGCCTGCTGATCTGCCTGCCATTGCT
GCTGATTTTGTTGAGGACCAGGAAGTGTGCAAGAACTATGCTGAGGCCAAGGATGTCTTCCT
GGGCACGTTCTTGTATGAATATTCAAGAAGACACCCTGATTACTCTGTATCCCTGTTGCTGA
GACTTGCTAAGAAATATGAAGCCACTCTGGAAAAGTGCTGCGCTGAAGCCAATCCTCCCGCA
TGCTACGGCACAGTGCTTGCTGAATTTCAGCCTCTTGTAGAAGAGCCTAAGAACTTGGTCAA
AACCAACTGTGATCTTTACGAGAAGCTTGGAGAATATGGATTCCAAAATGCCATTCTAGTTC
GCTACACCCAGAAAGCACCTCAGGTGTCAACCCCAACTCTCGTGGAGGCTGCAAGAAACCTA
GGAAGAGTGGGCACCAAGTGTTGTACACTTCCTGAAGATCAGAGACTGCCTTGTGTGGAAGA
CTATCTGTCTGCAATCCTGAACCGTGTGTGTCTGCTGCATGAGAAGACCCCAGTGAGTGAGC
ATGTTACCAAGTGCTGTAGTGGATCCCTGGTGGAAAGGCGGCCATGCTTCTCTGCTCTGACA
GTTGATGAAACATATGTCCCCAAAGAGTTTAAAGCTGAGACCTTCACCTTCCACTCTGATAT
CTGCACACTTCCAGAGAAGGAGAAGCAGATTAAGAAACAAACGGCTCTTGCTGAGCTGGTGA
AGCACAAGCCCAAGGCTACAGCGGAGCAACTGAAGACTGTCATGGATGACTTTGCACAGTTC
CTGGATACATGTTGCAAGGCTGCTGACAAGGACACCTGCTTCTCGACTGAGGGTCCAAACCT
TGTCACTAGATGCAAAGACGCCTTAGCCTGAGCGATCGCTAAATACAGCAGCAATTGGCAAG
CTGCTTACATAGAACTCGCGGCGATTGGCATGCCGCCTTAAAATTTTTATTTTATTTTTCTT
TTCTTTTCCGAATCGGATTTTGTTTTTAATATTTCAAAAAAAAAAAAAAAAAAAAAAAAAAC
GCGTCGAGGGGAATTAATTCTTGAAGACGAAAGGGCCAGGTGGCACTTTTCGGGGAAATGTG
CGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACA
ATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCC
GTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACG

CTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGA
TCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCA
CTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTC
GGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCA
TCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACA
CTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCAC
AACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACC
AAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAA
CTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAA
GTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGG
AGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCC
GTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATC
GCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATAT
ACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTG
ATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTA
GAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAC
AAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTC
CGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAG
TTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTT
ACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGT
TACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAG
CGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCC
CGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGA
GGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGA
CTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAA
CGCGAGCTCTAATACGACTCACTATAG (SEQ ID NO: 4)

[0062] In some instances', the replicon RNA-IL-12 is fused with a serum albumin coding sequence. In some instances, the IL-12-albumin fusion molecule has a longer half live in tumor microenvironment. In some instances, the IL-12-albumin fusion protein can persist in tumor microenvironment for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, or longer.

[0063] In some instances, the synthetic oncolytic virus comprises LNP-replicon RNA-IL-12-serum albumin. In other instances, the synthetic oncolytic virus comprises TT3-LNP-repliconRNA-IL-12-serum albumin. In other instances, the synthetic oncolytic virus comprises TT3-LNP-mtReplicon RNA-IL-12-serum albumin. The persist presence of IL-12 in the tumor microenvironment may prolong the anti-tumor immune response exerted by the synthetic oncolytic virus.

[0064] In some instances, the replicon RNA-IL12-serum albumin further comprises a coding sequence for lumican. Lumican is one of the major extracellular proteins in the interstitial extracellular matrix (ECM) of the skin, corneal stroma, sclera, aorta, muscle, lung, kidney, bone, cartilage and intervertebral discs. It is a member of the family of small, leucine-rich proteoglycans (SLRP), with a core protein of 30-50 kDa comprising a signal peptide, a negatively charged N-terminal domain, a highly conserved leucine-rich internal domain and a carboxyl-terminal domain. The protein core and the glycan chains of lumican can interact with various cellular effectors, including cytokines, growth factors and cell surface receptors, to modulate cell adhesion, proliferation and migration. As an endogenous collagen-binding protein, the presence of lumican in tumor microenvironment would promote local retention of IL-12 to enhance efficacy and safety of the tumor immunotherapy, as described herein. In some instances, the replicon RNA-IL-12-MSA comprises a coding sequence for lumican. In other instances, the mtReplicon RNA-IL-12-MSA (mtRep-IL12-MSA) comprises a coding sequence for lumican. An exemplary coding sequence for mtReplicon RNA-IL-12-MSA-lumican (mtRep-IL12-MSA-Lumican) is set forth in SEQ ID NO: 5:

ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAAATGGAGAAAGTTCACGTT
GACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGGAGCTTCCCGCAGTTTGAGGT
AGAAGCCAAGCAGGTCACTGATAATGACCATGCTAATGCCAGAGCGTTTTCGCATCTGGCTT
CAAAACTGATCGAAACGGAGGTGGACCCATCCGACACGATCCTTGACATTGGAAGTGCGCCC
GCCCGCAGAATGTATTCTAAGCACAAGTATCATTGTATCTGTCCGATGAGATGTGCGGAAGA
TCCGGACAGATTGTATAAGTATGCAACTAAGCTGAAGAAAAACTGTAAGGAAATAACTGATA
AGGAATTGGACAAGAAAATGAAGGAGCTGGCCGCCGTCATGAGCGACCCTGACCTGGAAACT
GAGACTATGTGCCTCCACGACGACGAGTCGTGTCGCTACGAAGGGCAAGTCGCTGTTTACCA
GGATGTATACGCGGTTGACGGACCGACAAGTCTCTATCACCAAGCCAATAAGGGAGTTAGAG
TCGCCTACTGGATAGGCTTTGACACCACCCCTTTTATGTTTAAGAACTTGGCTGGAGCATAT
CCATCATACTCTACCAACTGGGCCGACGAAACCGTGTTAACGGCTCGTAACATAGGCCTATG
CAGCTCTGACGTTATGGAGCGGTCACGTAGAGGGATGTCCATTCTTAGAAAGAAGTATTTGA
AACCATCCAACAATGTTCTATTCTCTGTTGGCTCGACCATCTACCACGAGAAGAGGGACTTA
CTGAGGAGCTGGCACCTGCCGTCTGTATTTCACTTACGTGGCAAGCAAAATTACACATGTCG
GTGTGAGACTATAGTTAGTTGCGACGGGTACGTCGTTAAAAGAATAGCTATCAGTCCAGGCC
TGTATGGGAAGCCTTCAGGCTATGCTGCTACGATGCACCGCGAGGGATTCTTGTGCTGCAAA
GTGACAGACACATTGAACGGGGAGAGGGTCTCTTTTCCCGTGTGCACGTATGTGCCAGCTAC
ATTGTGTGACCAAATGACTGGCATACTGGCAACAGATGTCAGTGCGGACGACGCGCAAAAAC
TGCTGGTTGGGCTCAACCAGCGTATAGTCGTCAACGGTCGCACCCAGAGAAACACCAATACC
ATGAAAAATTACCTTTTGCCCGTAGTGGCCCAGGCATTTGCTAGGTGGGCAAAGGAATATAA
GGAAGATCAAGAAGATGAAAGGCCACTAGGACTACGAGATAGACAGTTAGTCATGGGGTGTT
GTTGGGCTTTTAGAAGGCACAAGATAACATCTATTTATAAGCGCCCGGATACCCAAACCATC
ATCAAAGTGAACAGCGATTTCCACTCATTCGTGCTGCCCAGGATAGGCAGTAACACATTGGA
GATCGGGCTGAGAACAAGAATCAGGAAAATGTTAGAGGAGCACAAGGAGCCGTCACCTCTCA
TTACCGCCGAGGACGTACAAGAAGCTAAGTGCGCAGCCGATGAGGCTAAGGAGGTGCGTGAA
GCCGAGGAGTTGCGCGCAGCTCTACCACCTTTGGCAGCTGATGTTGAGGAGCCCACTCTGGA
AGCCGATGTCGACTTGATGTTACAAGAGGCTGGGGCCGGCTCAGTGGAGACACCTCGTGGCT
TGATAAAGGTTACCAGCTACGATGGCGAGGACAAGATCGGCTCTTACGCTGTGCTTTCTCCG
CAGGCTGTACTCAAGAGTGAAAAATTATCTTGCATCCACCCTCTCGCTGAACAAGTCATAGT
GATAACACACTCTGGCCGAAAAGGGCGTTATGCCGTGGAACCATACCATGGTAAAGTAGTGG
TGCCAGAGGGACATGCAATACCCGTCCAGGACTTTCAAGCTCTGAGTGAAAGTGCCACCATT
GTGTACAACGAACGTGAGTTCGTAAACAGGTACCTGCACCATATTGCCACACATGGAGGAGC
GCTGAACACTGATGAAGAATATTACAAAACTGTCAAGCCCAGCGAGCACGACGGCGAATACC
TGTACGACATCGACAGGAAACAGTGCGTCAAGAAAGAACTAGTCACTGGGCTAGGGCTCACA
GGCGAGCTGGTGGATCCTCCCTTCCATGAATTCGCCTACGAGAGTCTGAGAACACGACCAGC
CGCTCCTTACCAAGTACCAACCATAGGGGTGTATGGCGTGCCAGGATCAGGCAAGTCTGGCA
TCATTAAAAGCGCAGTCACCAAAAAAGATCTAGTGGTGAGCGCCAAGAAAGAAAACTGTGCA

GAAATTATAAGGGACGTCAAGAAAATGAAAGGGCTGGACGTCAATGCCAGAACTGTGGACTC
AGTGCTCTTGAATGGATGCAAACACCCCGTAGAGACCCTGTATATTGACGAAGCTTTTGCTT
GTCATGCAGGTACTCTCAGAGCGCTCATAGCCATTATAAGACCTAAAAAGGCAGTGCTCTGC
GGGGATCCCAAACAGTGCGGTTTTTTTAACATGATGTGCCTGAAAGTGCATTTTAACCACGA
GATTTGCACACAAGTCTTCCACAAAAGCATCTCTCGCCGTTGCACTAAATCTGTGACTTCGG
TCGTCTCAACCTTGTTTTACGACAAAAAAATGAGAACGACGAATCCGAAAGAGACTAAGATT
GTGATTGACACTACCGGCAGTACCAAACCTAAGCAGGACGATCTCATTCTCACTTGTTTCAG
AGGGTGGGTGAAGCAGTTGCAAATAGATTACAAAGGCAACGAAATAATGACGGCAGCTGCCT
CTCAAGGGCTGACCCGTAAAGGTGTGTATGCCGTTCGGTACAAGGTGAATGAAAATCCTCTG
TACGCACCCACCTCAGAACATGTGAACGTCCTACTGACCCGCACGGAGGACCGCATCGTGTG
GAAAACACTAGCCGGCGACCCATGGATAAAAACACTGACTGCCAAGTACCCTGGGAATTTCA
CTGCCACGATAGAGGAGTGGCAAGCAGAGCATGATGCCATCATGAGGCACATCTTGGAGAGA
CCGGACCCTACCGACGTCTTCCAGAATAAGGCAAACGTGTGTTGGGCCAAGGCTTTAGTGCC
GGTGCTGAAGACCGCTGGCATAGACATGACCACTGAACAATGGAACACTGTGGATTATTTTG
AAACGGACAAAGCTCACTCAGCAGAGATAGTATTGAACCAACTATGCGTGAGGTTCTTTGGA
CTCGATCTGGACTCCGGTCTATTTTCTGCACCCACTGTTCCGTTATCCATTAGGAATAATCA
CTGGGATAACTCCCCGTCGCCTAACATGTACGGGCTGAATAAAGAAGTGGTCCGTCAGCTCT
CTCGCAGGTACCCACAACTGCCTCGGGCAGTTGCCACTGGAAGAGTCTATGACATGAACACT
GGTACACTGCGCAATTATGATCCGCGCATAAACCTAGTACCTGTAAACAGAAGACTGCCTCA
TGCTTTAGTCCTCCACCATAATGAACACCCACAGAGTGACTTTTCTTCATTCGTCAGCAAAT
TGAAGGGCAGAACTGTCCTGGTGGTCGGGGAAAAGTTGTCCGTCCCAGGCAAAATGGTTGAC
TGGTTGTCAGACCGGCCTGAGGCTACCTTCAGAGCTCGGCTGGATTTAGGCATCCCAGGTGA
TGTGCCCAAATATGACATAATATTTGTTAATGTGAGGACCCCATATAAATACCATCACTATC
AGCAGTGTGAAGACCATGCCATTAAGCTTAGCATGTTGACCAAGAAAGCTTGTCTGCATCTG
AATCCCGGCGGAACCTGTGTCAGCATAGGTTATGGTTACGCTGACAGGGCCAGCGAAAGCAT
CATTGGTGCTATAGCGCGGCAGTTCAAGTTTTCCCGGGTATGCAAACCGAAATCCTCACTTG
AAGAGACGGAAGTTCTGTTTGTATTCATTCGGTACGATCGCAAGGCCCGTACGCACAATTCT
TACAAGCTTTCATCAACCTTGACCAACATTTATACAGGTTCCAGACTCCACGAAGCCGGATG
TGCACCCTCATATCATGTGGTGCGAGGGGATATTGCCACGGCCACCGAAGGAGTGATTATAA
ATGCTGCTAACAGCAAAGGACAACCTGGCGGAGGGGTGTGCGGAGCGCTGTATAAGAAATTC
CCGGAAAGCTTCGATTTACAGCCGATCGAAGTAGGAAAAGCGCGACTGGTCAAAGGTGCAGC
TAAACATATCATTCATGCCGTAGGACCAAACTTCAACAAAGTTTCGGAGGTTGAAGGTGACA
AACAGTTGGCAGAGGCTTATGAGTCCATCGCTAAGATTGTCAACGATAACAATTACAAGTCA
GTAGCGATTCCACTGTTGTCCACCGGCATCTTTTCCGGGAACAAAGATCGACTAACCCAATC
ATTGAACCATTTGCTGACAGCTTTAGACACCACTGATGCAGATGTAGCCATATACTGCAGGG
ACAAGAAATGGGAAATGACTCTCAAGGAAGCAGTGGCTAGGAGAGAAGCAGTGGAGGAGATA
TGCATATCCGACGACTCTTCAGTGACAGAACCTGATGCAGAGCTGGTGAGGGTGCATCCGAA
GAGTTCTTTGGCTGGAAGGAAGGGCTACAGCACAAGCGATGGCAAAACTTTCTCATATTTGG
AAGGGACCAAGTTTCACCAGGCGGCCAAGGATATAGCAGAAATTAATGCCATGTGGCCCGTT
GCAACGGAGGCCAATGAGCAGGTATGCATGTATATCCTCGGAGAAGGCATGAGCAGTATTAG
GTCGAAATGCCCCGTCGAAGAGTCGGAAGCCTCCACACCACCTAGCACGCTGCCTTGCTTGT
GCATCCATGCCATGACTCCAGAAAGAGTACAGCGCCTAAAAGCCTCACGTCCAGAACAAATT
ACTGTGTGCTCATCCTTTCCATTGCCGAAGTATAGAATCACTGGTGTGCAGAAGATCCAATG
CTCCCAGCCTATATTGTTCTCACCGAAAGTGCCTGCGTATATTCATCCAAGGAAGTATCTCG
TGGAAACACCACCGGTAGACGAGACTCCGGAGCCATCGGCAGAGAACCAATCCACAGAGGGG
ACACCTGAACAACCACCACTTATAACCGAGGATGAGACCAGGACTAGAACGCCTGAGCCGAT
CATCATCGAAGAGGAAGAAGAGGATAGCATAAGTTTGCTGTCAGATGGCCCGACCCACCAGG
TGCTGCAAGTCGAGGCAGACATTCACGGGCCGCCCTCTGTATCTAGCTCATCCTGGTCCATT
CCTCATGCATCCGACTTTGATGTGGACAGTTTATCCATACTTGACACCCTGGAGGGAGCTAG
CGTGACCAGCGGGGCAACGTCAGCCGAGACTAACTCTTACTTCGCAAAGAGTATGGAGTTTC
TGGCGCGACCGGTGCCTGCGCCTCGAACAGTATTCAGGAACCCTCCACATCCCGCTCCGCGC
ACAAGAACACCGTCACTTGCACCCAGCAGGGCCTGCTCGAGAACCAGCCTAGTTTCCACCCC

GCCAGGCGTGAATAGGGTGATCACTAGAGAGGAGCTCGAGGCGCTTACCCCGTCACGCACTC
CTAGCAGGTCGGTCTCGAGAACCAGCCTGGTCTCCAACCCGCCAGGCGTAAATAGGGTGATT
ACAAGAGAGGAGTTTGAGGCGTTCGTAGCACAACAACAATGACGGTTTGATGCGGGTGCATA
CATCTTTTCCTCCGACACCGGTCAAGGGCATTTACAACAAAAATCAGTAAGGCAAACGGTGC
TATCCGAAGTGGTGTTGGAGAGGACCGAATTGGAGATTTCGTATGCCCCGCGCCTCGACCAA
GAAAAAGAAGAATTACTACGCAAGAAATTACAGTTAAATCCCACACCTGCTAACAGAAGCAG
ATACCAGTCCAGGAAGGTGGAGAACATGAAAGCCATAACAGCTAGACGTATTCTGCAAGGCC
TAGGGCATTATTTGAAGGCAGAAGGAAAAGTGGAGTGCTACCGAACCCTGCATCCTGTTCCT
TTGTATTCATCTAGTGTGAACCGTGCCTTTTCAAGCCCCAAGGTCGCAGTGGAAGCCTGTAA
CGCCATGTTGAAAGAGAACTTTCCGACTGTGGCTTCTTACTGTATTATTCCAGAGTACGATG
CCTATTTGGACATGGTTGACGGAGCTTCATGCTGCTTAGACACTGCCAGTTTTTGCCCTGCA
AAGCTGCGCAGCTTTCCAAAGAAACACTCCTATTTGGAACCCACAATACGATCGGCAGTGCC
TTCAGCGATCCAGAACACGCTCCAGAACGTCCTGGCAGCTGCCACAAAAAGAAATTGCAATG
TCACGCAAATGAGAGAATTGCCCGTATTGGATTCGGCGGCCTTTAATGTGGAATGCTTCAAG
AAATATGCGTGTAATAATGAATATTGGGAAACGTTTAAAGAAAACCCCATCAGGCTTACTGA
AGAAAACGTGGTAAATTACATTACCAAATTAAAAGGACCAAAAGCTGCTGCTCTTTTTGCGA
AGACACATAATTTGAATATGTTGCAGGACATACCAATGGACAGGTTTGTAATGGACTTAAAG
AGAGACGTGAAAGTGACTCCAGGAACAAAACATACTGAAGAACGGCCCAAGGTACAGGTGAT
CCAGGCTGCCGATCCGCTAGCAACAGCGTATCTGTGCGGAATCCACCGAGAGCTGGTTAGGA
GATTAAATGCGGTCCTGCTTCCGAACATTCATACACTGTTTGATATGTCGGCTGAAGACTTT
GACGCTATTATAGCCGAGCACTTCCAGCCTGGGGATTGTGTTCTGGAAACTGACATCGCGTC
GTTTGATAAAAGTGAGGACGACGCCATGGCTCTGACCGCGTTAATGATTCTGGAAGACTTAG
GTGTGGACGCAGAGCTGTTGACGCTGATTGAGGCGGCTTTCGGCGAAATTTCATCAATACAT
TTGCCCACTAAAACTAAATTTAAATTCGGAGCCATGATGAAATCTGGAATGTTCCTCACACT
GTTTGTGAACACAGTCATTAACATTGTAATCGCAAGCAGAGTGTTGAGAGAACGGCTAACCG
GATCACCATGTGCAGCATTCATTGGAGATGACAATATCGTGAAAGGAGTCAAATCGGACAAA
TTAATGGCAGACAGGTGCGCCACCTGGTTGAATATGGAAGTCAAGATTATAGATGCTGTGGT
GGGCGAGAAAGCGCCTTATTTCTGTGGAGGGTTTATTTTGTGTGACTCCGTGACCGGCACAG
CGTGCCGTGTGGCAGACCCCCTAAAAAGGCTGTTTAAGCTTGGCAAACCTCTGGCAGCAGAC
GATGAACATGATGATGACAGGAGAAGGGCATTGCATGAAGAGTCAACACGCTGGAACCGAGT
GGGTATTCTTTCAGAGCTGTGCAAGGCAGTAGAATCAAGGTATGAAACCGTAGGAACTTCCA
TCATAGTTATGGCCATGACTACTCTAGCTAGCAGTGTTAAATCATTCAGCTACCTGAGAGGG
GCCCCTATAACTCTCTACGGCTAACCTGAATGGACTACGACATAGTCTAGTCCGCCAAGTCT
AGCATATGGGCGCGCCCTCAGCATCGATTTGAATTGGCCACCATGAGGGTCCCCGCTCAGCT
CCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCACGATGTGCCATGTGGGAGCTGGAGAAAG
ACGTTTATGTTGTAGAGGTGGACTGGACTCCCGATGCCCCTGGAGAAACAGTGAACCTCACC
TGTGACACGCCTGAAGAAGATGACATCACCTGGACCTCAGACCAGAGACATGGAGTCATAGG
CTCTGGAAAGACCCTGACCATCACTGTCAAAGAGTTTCTAGATGCTGGCCAGTACACCTGCC
ACAAAGGAGGCGAGACTCTGAGCCACTCACATCTGCTGCTCCACAAGAAGGAAAATGGAATT
TGGTCCACTGAAATTTTAAAAAATTTCAAAAACAAGACTTTCCTGAAGTGTGAAGCACCAAA
TTACTCCGGACGGTTCACGTGCTCATGGCTGGTGCAAAGAAACATGGACTTGAAGTTCAACA
TCAAGAGCAGTAGCAGTTCCCCTGACTCTCGGGCAGTGACATGTGGAATGGCGTCTCTGTCT
GCAGAGAAGGTCACACTGGACCAAAGGGACTATGAGAAGTATTCAGTGTCCTGCCAGGAGGA
TGTCACCTGCCCAACTGCCGAGGAGACCCTGCCCATTGAACTGGCGTTGGAAGCACGGCAGC
AGAATAAATATGAGAACTACAGCACCAGCTTCTTCATCAGGGACATCATCAAACCAGACCCG
CCCAAGAACTTGCAGATGAAGCCTTTGAAGAACTCACAGGTGGAGGTCAGCTGGGAGTACCC
TGACTCCTGGAGCACTCCCCATTCCTACTTCTCCCTCAAGTTCTTTGTTCGAATCCAGCGCA
AGAAAGAAAAGATGAAGGAGACAGAGGAGGGGTGTAACCAGAAAGGTGCGTTCCTCGTAGAG
AAGACATCTACCGAAGTCCAATGCAAAGGCGGGAATGTCTGCGTGCAAGCTCAGGATCGCTA
TTACAATTCCTCATGCAGCAAGTGGGCATGTGTTCCCTGCAGGGTCCGATCCGGAGGTTCCG
GTGGTGGATCCGGAGGTGGCTCCGGCGGCGGATCCAGGGTCATTCCAGTCTCTGGACCTGCC
AGGTGTCTTAGCCAGTCCCGAAACCTGCTGAAGACCACAGATGACATGGTGAAGACGGCCAG

AGAAAAACTGAAACATTATTCCTGCACTGCTGAAGACATCGATCATGAAGACATCACACGGG
ACCAAACCAGCACATTGAAGACCTGTTTACCACTGGAACTACACAAGAACGAGAGTTGCCTG
GCTACTAGAGAGACTTCTTCCACAACAAGAGGGAGCTGCCTGCCCCCACAGAAGACGTCTTT
GATGATGACCCTGTGCCTTGGTAGCATCTATGAGGACTTGAAGATGTACCAGACAGAGTTCC
AGGCCATCAACGCAGCACTTCAGAATCACAACCATCAGCAGATCATTCTAGACAAGGGCATG
CTGGTGGCCATCGATGAGCTGATGCAGTCTCTGAATCATAATGGCGAGACTCTGCGCCAGAA
ACCTCCTGTGGGAGAAGCAGACCCTTACAGAGTGAAAATGAAGCTCTGCATCCTGCTTCACG
CCTTCAGCACCCGCGTCGTGACCATCAACAGGGTGATGGGCTATCTGAGCTCCGCCGGTTCC
GGTGGCGGATCCGAAGCACACAAGAGTGAGATCGCCCATCGGTATAATGATTTGGGAGAACA
ACATTTCAAAGGCCTAGTCCTGATTGCCTTTTCCCAGTATCTCCAGAAATGCTCATACGATG
AGCATGCCAAATTAGTGCAGGAAGTAACAGACTTTGCAAAGACGTGTGTTGCCGATGAGTCT
GCCGCCAACTGTGACAAATCCCTTCACACTCTTTTTGGAGATAAGTTGTGTGCCATTCCAAA
CCTCCGTGAAAACTATGGTGAACTGGCTGACTGCTGTACAAAACAAGAGCCCGAAAGAAACG
AATGTTTCCTGCAACACAAAGATGACAACCCCAGCCTGCCACCATTTGAAAGGCCAGAGGCT
GAGGCCATGTGCACCTCCTTTAAGGAAAACCCAACCACCTTTATGGGACACTATTTGCATGA
AGTTGCCAGAAGACATCCTTATTTCTATGCCCCAGAACTTCTTTACTATGCTGAGCAGTACA
ATGAGATTCTGACCCAGTGTTGTGCAGAGGCTGACAAGGAAAGCTGCCTGACCCCGAAGCTT
GATGGTGTGAAGGAGAAAGCATTGGTCTCATCTGTCCGTCAGAGAATGAAGTGCTCCAGTAT
GCAGAAGTTTGGAGAGAGAGCTTTTAAAGCATGGGCAGTAGCTCGTCTGAGCCAGACATTCC
CCAATGCTGACTTTGCAGAAATCACCAAATTGGCAACAGACCTGACCAAAGTCAACAAGGAG
TGCTGCCATGGTGACCTGCTGGAATGCGCAGATGACAGGGCGGAACTTGCCAAGTACATGTG
TGAAAACCAGGCGACTATCTCCAGCAAACTGCAGACTTGCTGCGATAAACCACTGTTGAAGA
AAGCCCACTGTCTTAGTGAGGTGGAGCATGACACCATGCCTGCTGATCTGCCTGCCATTGCT
GCTGATTTTGTTGAGGACCAGGAAGTGTGCAAGAACTATGCTGAGGCCAAGGATGTCTTCCT
GGGCACGTTCTTGTATGAATATTCAAGAAGACACCCTGATTACTCTGTATCCCTGTTGCTGA
GACTTGCTAAGAAATATGAAGCCACTCTGGAAAAGTGCTGCGCTGAAGCCAATCCTCCCGCA
TGCTACGGCACAGTGCTTGCTGAATTTCAGCCTCTTGTAGAAGAGCCTAAGAACTTGGTCAA
AACCAACTGTGATCTTTACGAGAAGCTTGGAGAATATGGATTCCAAAATGCCATTCTAGTTC
GCTACACCCAGAAAGCACCTCAGGTGTCAACCCCAACTCTCGTGGAGGCTGCAAGAAACCTA
GGAAGAGTGGGCACCAAGTGTTGTACACTTCCTGAAGATCAGAGACTGCCTTGTGTGGAAGA
CTATCTGTCTGCAATCCTGAACCGTGTGTGTCTGCTGCATGAGAAGACCCCAGTGAGTGAGC
ATGTTACCAAGTGCTGTAGTGGATCCCTGGTGGAAAGGCGGCCATGCTTCTCTGCTCTGACA
GTTGATGAAACATATGTCCCCAAAGAGTTTAAAGCTGAGACCTTCACCTTCCACTCTGATAT
CTGCACACTTCCAGAGAAGGAGAAGCAGATTAAGAAACAAACGGCTCTTGCTGAGCTGGTGA
AGCACAAGCCCAAGGCTACAGCGGAGCAACTGAAGACTGTCATGGATGACTTTGCACAGTTC
CTGGATACATGTTGCAAGGCTGCTGACAAGGACACCTGCTTCTCGACTGAGGGTCCAAACCT
TGTCACTAGATGCAAAGACGCCTTAGCCGGCGGAGGTTCCGGTGGCGGATCCCAATACTATG
ACTACGATATACCCCTGTTCATGTACGGGCAAATATCTCCAAACTGTGCACCAGAATGTAAC
TGCCCTCACTCATACCCCACTGCAATGTACTGTGACGACCTGAAGTTGAAATCCGTGCCAAT
GGTGCCTCCTGGGATTAAGTACCTGTACCTCCGCAACAATCAGATCGACCATATTGACGAGA
AGGCTTTTGAAAACGTCACAGACCTCCAGTGGCTTATCCTGGACCATAACCTGCTTGAAAAT
AGTAAGATAAAGGGCAAAGTATTTTCCAAACTTAAACAGCTTAAAAAACTCCACATCAACTA
CAATAACCTTACTGAATCCGTGGGACCATTGCCAAAATCTCTCCAAGATTTGCAGTTGACTA
ACAACAAGATATCCAAACTCGGCTCCTTCGATGGGCTGGTTAATCTGACTTTCATCTACTTG
CAACACAACCAATTGAAGGAGGATGCAGTTTCAGCTAGTCTTAAAGGTCTGAAAAGCCTTGA
GTATCTTGATCTGTCATTTAATCAAATGTCCAAGCTCCCTGCTGGGCTCCCAACAAGTCTGC
TGACACTCTATCTCGACAATAACAAGATAAGTAACATTCCCGATGAGTACTTTAAAAGATTT
ACCGGCCTCCAATACTTGCGGCTTTCTCACAACGAGTTGGCAGACTCTGGTGTACCCGGCAA
CTCCTTTAATATAAGTTCTCTTCTCGAGCTTGATTTGTCCTATAACAAACTGAAGAGTATCC
CTACTGTCAATGAAAATTTGGAGAATTACTACCTCGAAGTCAATGAGCTTGAGAAGTTCGAT
GTTAAGTCTTTCTGTAAGATACTGGGTCCATTGTCATACAGCAAGATTAAACATCTTCGCTT
GGATGGGAATCCCTTGACTCAAAGCTCACTTCCCCCCGACATGTACGAATGCCTGAGGGTAG

CCAACGAAATCACAGTAAACGGAGGTGGCTCCTGAGCGATCGCTAAATACAGCAGCAATTGG
CAAGCTGCTTACATAGAACTCGCGGCGATTGGCATGCCGCCTTAAAATTTTTATTTTATTTT
TCTTTTCTTTTCCGAATCGGATTTTGTTTTTAATATTTCAAAAAAAAAAAAAAAAAAAAAA
AAACGCGTCGAGGGGAATTAATTCTTGAAGACGAAAGGGCCAGGTGGCACTTTTCGGGGAAA
TGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
GACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACAT
TTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGA
AACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAAC
TGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATG
AGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCA
ACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAA
AGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGAT
AACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTT
GCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCA
TACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTA
TTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGA
TAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAAT
CTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCC
TCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACA
GATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCAT
ATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTT
TTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCC
CGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGC
AAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTT
TTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCC
GTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCC
TGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGA
TAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTT
GGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGC
TTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGC
ACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCT
CTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCA
GCAACGCGAGCTCTAATACGACTCACTATAG (SEQ ID NO: 5)

[0065] In some instances, the synthetic oncolytic virus comprises LNP-replicon RNA-IL-12-serum albumin-lumican. In other instances, the synthetic oncolytic virus comprises TT3-LNP-repliconRNA-IL-12-serum albumin-lumican. In other instances, the synthetic oncolytic virus comprises TT3-LNP-mtReplicon RNA-IL-12-serum albumin-lumican. The retention of IL-12 in the tumor microenvironment may improve the efficacy and safety of the synthetic oncolytic virus.

[0066] In some instances, the replicon RNA may comprise one or more gene(s) of experimental or therapeutic interest. In some instances, the gene(s) of experimental or therapeutic interest encode cytokines, chemokines, or growth factors other than IL-12. Cytokines are known in the art, and the term itself refers to a generalized grouping of small proteins that are secreted by certain cells within the immune system and have an effect on other cells. Cytokines are known to enhance the cellular immune response and, as used herein, can include, but are not limited to, TNFα, IFN-γ, IFN-α, TGF-β, IL-1, IL-2, IL-4, IL-10, IL-13, IL-17, IL-18, and chemokines. Chemokines are useful for studies investigating response to infection, immune responses, inflammation, trauma, sepsis, cancer, and reproduction, among other applications. Chemokines are known in the art, and are a type of cytokines that induce chemotaxis in nearby responsive cells, typically of white blood cells, to sites of infection. Non-limiting examples of chemokines include, CCL14, CCL19, CCL20, CCL21, CCL25, CCL27, CXCL12, CXCL13, CXCL-8, CCL2, CCL3, CCL4, CCL5, CCL11, and CXCL10. Growth factors are known in the art, and the term itself is sometimes interchangeable with the term cytokines. As used herein, the term "growth factors" refers to a naturally occurring substance capable of signaling between cells and stimulating cellular growth. While cytokines may be growth factors, certain types of cytokines may also have an inhibitory effect on cell growth, thus differentiating the two terms. Non-limiting examples of growth factors include Adrenomedullin (AM), Angiopoietin (Ang), Autocrine motility factor, Bone morphogenetic proteins (BMPs), Ciliary neurotrophic factor (CNTF), Leukemia inhibitory

factor (LIF), Interleukin-6 (IL-6), Macrophage colony-stimulating factor (m-CSF), Granulocyte colony-stimulating factor (G-CSF), Granulocyte macrophage colony-stimulating factor (GM-CSF), Epidermal growth factor (EGF), Ephrin A1, Ephrin A2, Ephrin A3, Ephrin A4, Ephrin A5, Ephrin B1, Ephrin B2, Ephrin B3, Erythropoietin (EPO), Fibroblast growth factor 1(FGF1), Fibroblast growth factor 2(FGF2), Fibroblast growth factor 3(FGF3), Fibroblast growth factor 4(FGF4), Fibroblast growth factor 5(FGF5), Fibroblast growth factor 6(FGF6), Fibroblast growth factor 7(FGF7), Fibroblast growth factor 8(FGF8), Fibroblast growth factor 9(FGF9), Fibroblast growth factor 10(FGF10), Fibroblast growth factor 11(FGF11), Fibroblast growth factor 12(FGF12), Fibroblast growth factor 13(FGF13), Fibroblast growth factor 14(FGF14), Fibroblast growth factor 15(FGF15), Fibroblast growth factor 16(FGF16), Fibroblast growth factor 17(FGF17), Fibroblast growth factor 18(FGF18), Fibroblast growth factor 19(FGF19), Fibroblast growth factor 20(FGF20), Fibroblast growth factor 21(FGF21), Fibroblast growth factor 22(FGF22), Fibroblast growth factor 23(FGF23), Fetal Bovine Somatotrophin (FBS), Glial cell line-derived neurotrophic factor (GDNF), Neurturin, Persephin, Artemin, Growth differentiation factor-9 (GDF9), Hepatocyte growth factor (HGF), Hepatoma-derived growth factor (HDGF), Insulin, Insulin-like growth factor-1 (IGF-1), Insulin-like growth factor-2 (IGF-2), Interleukin-1 (IL-1),IL-2 ,IL-3, IL-4, IL-5, IL-6, IL-7, Keratinocyte growth factor (KGF), Migration-stimulating factor (MSF), Macrophage-stimulating protein (MSP), Myostatin (GDF-8), Neuregulin 1 (NRG1), Neuregulin 2 (NRG2), Neuregulin 3 (NRG3), Neuregulin 4 (NRG4), Brain-derived neurotrophic factor (BDNF), Nerve growth factor (NGF), Neurotrophin-3 (NT-3), Neurotrophin-4 (NT-4), Placental growth factor (PGF), Platelet-derived growth factor (PDGF), Renalase (RNLS), T-cell growth factor (TCGF), Thrombopoietin (TPO), Transforming growth factor alpha (TGF-α), Transforming growth factor beta (TGF-β), Tumor necrosis factor-alpha (TNF-α), and Vascular endothelial growth factor (VEGF).

II. Pharmaceutical compositions

[0067] In some aspects, the present disclosure, at least in part, relates to a pharmaceutical composition, comprising the synthetic oncolytic virus, as described herein. The pharmaceutical composition described herein may further comprise a pharmaceutically acceptable carrier (excipient) to form a pharmaceutical composition for use in treating a target disease. "Acceptable" means that the carrier must be compatible with the active ingredient of the composition (and preferably, capable of stabilizing the active ingredient) and not deleterious to the subject to be treated. Pharmaceutically acceptable excipients (carriers) including buffers, which are well known in the art. See, e.g., Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover.

[0068] The pharmaceutical compositions to be used for in vivo administration must be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes. The synthetic oncolytic virus-containing compositions may be placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0069] In some instances, the pharmaceutical composition to be used herein, can be formulated for intratumoral injection. Intratumoral injection, as used herein, refers to direct injections into the tumor, an anti-tumor composition (e.g. immunostimulatory synthetic oncolytic virus). A high concentration of composition can be achieved in situ, while using small amounts of drugs. Local delivery of immunotherapies allows multiple combination therapies, while preventing significant systemic exposure and off-target toxicities.

[0070] In other instances, the pharmaceutical composition can be formulated for intramuscular injection, intravenous injection, or subcutaneous injection.

[0071] The pharmaceutical compositions to be used in the present methods can comprise pharmaceutically acceptable carriers, buffer agents, excipients, salts, or stabilizers in the form of lyophilized formulations or aqueous solutions. See, *e.g.,* Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations used, and may comprise buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0072] In some examples, the pharmaceutical composition described herein comprises lipid nanoparticles which can be prepared by methods known in the art, such as described in Epstein, et al., Proc. Natl. Acad. Sci. USA 82:3688 (1985); Hwang, et al., Proc. Natl. Acad. Sci. USA 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-

derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

[0073] In other examples, the pharmaceutical composition described herein can be formulated in sustained-release format. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the synthetic oncolytic virus which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

[0074] Suitable surface-active agents include, in particular, non-ionic agents, such as polyoxyethylenesorbitans (*e.g.,* TWEEN™ 20, 40, 60, 80 or 85) and other sorbitans (*e.g.,* SPAN™ 20, 40, 60, 80 or 85). Compositions with a surface-active agent will conveniently comprise between 0.05 and 5% surface-active agent, and can be between 0.1 and 2.5%. It will be appreciated that other ingredients may be added, for example mannitol or other pharmaceutically acceptable vehicles, if necessary.

[0075] The pharmaceutical compositions described herein can be in unit dosage forms such as tablets, pills, capsules, powders, granules, solutions or suspensions, or suppositories, for oral, parenteral or rectal administration, or administration by inhalation or insufflation.

[0076] For preparing solid compositions such as tablets, the principal active ingredient can be mixed with a pharmaceutical carrier, e.g., conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other pharmaceutical diluents, e.g., water, to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention, or a non-toxic pharmaceutically acceptable salt thereof. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type described above containing from 0.1 to about 500 mg of the active ingredient of the present invention. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer that serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

[0077] Suitable emulsions may be prepared using commercially available fat emulsions, such as INTRALIPID™, LIPOSYN™, INFONUTROL™, LIPOFUNDIN™ and LIPIPHYSAN™. The active ingredient may be either dissolved in a pre-mixed emulsion composition or alternatively it may be dissolved in an oil (e.g., soybean oil, safflower oil, cottonseed oil, sesame oil, corn oil or almond oil) and an emulsion formed upon mixing with a phospholipid (*e.g.*, egg phospholipids, soybean phospholipids or soybean lecithin) and water. It will be appreciated that other ingredients may be added, for example glycerol or glucose, to adjust the tonicity of the emulsion. Suitable emulsions will typically contain up to 20% oil, for example, between 5 and 20%. The fat emulsion can comprise fat droplets having a suitable size and can have a pH in the range of 5.5 to 8.0.

[0078] Pharmaceutical compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as set out above. In some instances, the compositions are administered by the oral or nasal respiratory route for local or systemic effect.

[0079] Compositions in preferably sterile pharmaceutically acceptable solvents may be nebulized by use of gases. Nebulized solutions may be breathed directly from the nebulizing device or the nebulizing device may be attached to a face mask, tent or intermittent positive pressure breathing machine. Solution, suspension or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

III. Therapeutic Applications

[0080] The pharmaceutical compositions disclosed herein, comprising a synthetic oncolytic virus, can be used to treat cancer, for example, cancer immunotherapy.

[0081] To practice the method disclosed herein, an effective amount of any of the pharmaceutical compositions described herein can be administered to a subject (e.g., a human) in need of the treatment via a suitable route, such as intratumoral administration, by intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral,

inhalation or topical routes. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be directly nebulized and lyophilized powder can be nebulized after reconstitution. Alternatively, synthetic oncolytic virus containing pharmaceutical composition can be aerosolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder. In some examples, the pharmaceutical composition described herein is formulated for intratumoral injection. In particular examples, the pharmaceutical composition may be administered to a subject (e.g., a human patient) via a local route, for example, injected to a local site such as a tumor site or an infectious site.

[0082] As used herein, "an effective amount" refers to the amount of each active agent required to confer therapeutic effect on the subject, either alone or in combination with one or more other active agents. In some instances, the therapeutic effect is reduced tumor burden, reduction of cancer cells, or increased immune activity. Determination of whether an amount of synthetic oncolytic virus achieved the therapeutic effect would be evident to one of skill in the art. Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment.

[0083] Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. Frequency of administration may be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of a target disease/disorder. Alternatively, sustained continuous release formulations of a synthetic oncolytic virus may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

[0084] In some instances, the treatment is a single injection of the synthetic oncolytic virus containing pharmaceutical composition. In some instances, the single injection is administered intratumorally to the subject in need thereof.

[0085] In some example, dosages for a synthetic oncolytic virus as described herein may be determined empirically in individuals who have been given one or more administration(s) of synthetic oncolytic. Individuals are given incremental dosages of the synthetic oncolytic containing composition. To assess efficacy of the synthetic oncolytic virus, an indicator of the disease/disorder can be followed. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of symptoms occurs or until sufficient therapeutic levels are achieved to alleviate a target disease or disorder, or a symptom thereof.

[0086] In some instances, dosing frequency is once every week, every 2 weeks, every 4 weeks, every 5 weeks, every 6 weeks, every 7 weeks, every 8 weeks, every 9 weeks, or every 10 weeks; or once every month, every 2 months, or every 3 months, or longer. The progress of this therapy is easily monitored by conventional techniques and assays. The dosing regimen of the synthetic oncolytic virus used can vary over time.

[0087] In some instances, the method described herein comprises administering to a subject in need of the treatment (e.g., a human patient) one or multiple doses of synthetic oncolytic virus-containing pharmaceutical composition.

[0088] For the purpose of the present disclosure, the appropriate dosage synthetic oncolytic virus as described herein will depend on the specific synthetic oncolytic virus, the type and severity of the disease/disorder, the synthetic oncolytic virus is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the synthetic oncolytic virus, and the discretion of the attending physician. A clinician may administer a synthetic oncolytic virus, until a dosage is reached that achieves the desired result. In some instances, the desired result is a decrease in tumor burden, a decrease in cancer cells, or increased immune activity. Methods of determining whether a dosage resulted in the desired result would be evident to one of skill in the art. Administration of one or more synthetic oncolytic virus can be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration synthetic oncolytic virus may be essentially continuous over a preselected period of time or may be in a series of spaced dose, e.g., either before, during, or after developing a target disease or disorder.

[0089] As used herein, the term "treating" refers to the application or administration of a composition including one or more active agents to a subject, who has a target disease or disorder, a symptom of the disease/disorder, or a predisposition toward the disease/disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disorder, the symptom of the disease, or the predisposition toward the disease or disorder.

[0090] Alleviating a target disease/disorder includes delaying the development or progression of the disease, or reducing disease severity. Alleviating the disease does not necessarily require curative results. As used therein, "delaying" the development of a target disease or disorder means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. A method that "delays" or alleviates the development of a disease, or delays the onset of the disease, is a method that reduces probability of developing one or more symptoms of the disease in a given time frame and/or reduces extent of

the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a statistically significant result.

[0091] "Development" or "progression" of a disease means initial manifestations and/or ensuing progression of the disease. Development of the disease can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, development or progression refers to the biological course of the symptoms. "Development" includes occurrence, recurrence, and onset. As used herein "onset" or "occurrence" of a target disease or disorder includes initial onset and/or recurrence.

[0092] In some instances, a synthetic oncolytic virus-containing pharmaceutical composition as described herein are administered to a subject in need of the treatment at an amount sufficient to reduce tumor burden or cancer cell growth, by at least 5% (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater) in vivo. In other instances, the synthetic oncolytic virus-containing pharmaceutical compositions as described herein can be administered in an amount effective in increasing immune activity by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater.

[0093] The subject to be treated by the methods described herein can be a mammal, such as a human, farm animals, sport animals, pets, primates, horses, dogs, cats, mice and rats. In one instance, the subject is a human. The synthetic oncolytic virus-containing composition as described herein may be used for enhancing immune activity, for example, Tcell activity, in a subject in need of the treatment.

[0094] In some instances, the subject may be a human patient having, suspected of having, or at risk for a cancer. Non limiting examples of cancers include melanoma, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, gastric cancer, and various types of head and neck cancer, including squamous cell head and neck cancer. In some instances, the cancer can be melanoma, lung cancer, colorectal cancer, renal-cell cancer, urothelial carcinoma, or Hodgkin's lymphoma.

[0095] A subject having a target disease or disorder (e.g., cancer) can be identified by routine medical examination, e.g., laboratory tests, organ functional tests, CT scans, or ultrasounds. A subject suspected of having any of such target disease/disorder might show one or more symptoms of the disease/disorder. A subject at risk for the disease/disorder can be a subject having one or more of the risk factors associated with that disease/disorder. Such a subject can also be identified by routine medical practices.

[0096] In some instances, a synthetic oncolytic virus-containing pharmaceutical composition may be co-used with another suitable therapeutic agent (e.g., an anti-cancer agent an antiviral agent, or an anti-bacterial agent) and/or other agents that serve to enhance and/or complement the immunostimulatory effect of a synthetic oncolytic virus. In such combined therapy, the a synthetic oncolytic virus-containing composition and the additional therapeutic agent (e.g., an anti-cancer therapeutic agent or others described herein) may be administered to a subject in need of the treatment in a sequential manner, i.e., each therapeutic agent is administered at a different time. Alternatively, these therapeutic agents, or at least two of the agents, are administered to the subject in a substantially simultaneous manner.

[0097] Combination therapy can also embrace the administration of the agents described herein (e.g., a synthetic oncolytic virus containing pharmaceutical composition and an anti-cancer agent) in further combination with other biologically active ingredients (e.g., a different anti-cancer agent) and non-drug therapies (e.g., surgery).

[0098] It should be appreciated that any combination of a synthetic oncolytic virus - containing composition and another anti-cancer agent (e.g., a chemotherapeutic agent) may be used in any sequence for treating a cancer. The combinations described herein may be selected on the basis of a number of factors, which include but are not limited to the effectiveness of reducing tumor formation or tumor growth, reducing cancer cells, increasing immune activity, and/or alleviating at least one symptom associated with the cancer, or the effectiveness for mitigating the side effects of another agent of the combination. For example, a combined therapy described herein may reduce any of the side effects associated with each individual members of the combination, for example, a side effect associated with the anti-cancer agent.

[0099] In some instances, another anti-cancer therapeutic agent is a chemotherapy, a radiation therapy, a surgical therapy and/or an immunotherapy. Examples of the chemotherapeutic agents include, but are not limited to, Carboplatin or Cisplatin, Docetaxel, Gemcitabine, Nab-Paclitaxel, Paclitaxel, Pemetrexed, and Vinorelbine. Examples of radiation therapy include, but are not limited to, ionizing radiation, gamma-radiation, neutron beam radiotherapy, electron beam radiotherapy, proton therapy, brachytherapy, systemic radioactive isotopes and radiosensitizers. Examples of a surgical therapy include, but are not limited to, a curative surgery (e.g., tumor removal surgery), a preventive surgery, a laparoscopic surgery, and a laser surgery. Examples of an immunotherapy include, but are not limited to, adoptive cell transfer and therapeutic cancer vaccines.

[0100] Additional examples of chemotherapy include, but are not limited to, platinating agents, such as Carboplatin, Oxaliplatin, Cisplatin, Nedaplatin, Satraplatin, Lobaplatin, Triplatin, Tetranitrate, Picoplatin, Prolindac, Aroplatin and other derivatives; Topoisomerase I inhibitors, such as Camptothecin, Topotecan, irinotecan/SN38, rubitecan, Belotecan, and

other derivatives; Topoisomerase II inhibitors, such as Etoposide (VP-16), Daunorubicin, a doxorubicin agent (e.g., doxorubicin, doxorubicin HCl, doxorubicin analogs, or doxorubicin and salts or analogs thereof in liposomes), Mitoxantrone, Aclarubicin, Epirubicin, Idarubicin, Amrubicin, Amsacrine, Pirarubicin, Valrubicin, Zorubicin, Teniposide and other derivatives; Antimetabolites, such as Folic family (Methotrexate, Pemetrexed, Raltitrexed, Aminopterin, and relatives); Purine antagonists (Thioguanine, Fludarabine, Cladribine, 6-Mercaptopurine, Pentostatin, clofarabine and relatives) and Pyrimidine antagonists (Cytarabine, Floxuridine, Azacitidine, Tegafur, Carmofur, Capacitabine, Gemcitabine, hydroxyurea, 5-Fluorouracil (5FU), and relatives); Alkylating agents, such as Nitrogen mustards (e.g., Cyclophosphamide, Melphalan, Chlorambucil, mechlorethamine, Ifosfamide, Trofosfamide, Prednimustine, Bendamustine, Uramustine, Estramustine, and relatives); nitrosoureas (e.g., Carmustine, Lomustine, Semustine, Fotemustine, Nimustine, Ranimustine, Streptozocin, and relatives); Triazenes (e.g., Dacarbazine, Altretamine, Temozolomide, and relatives); Alkyl sulphonates (e.g., Busulfan, Mannosulfan, Treosulfan, and relatives); Procarbazine; Mitobronitol, and Aziridines (e.g., Carboquone, Triaziquone, ThioTEPA, triethylenemalamine, and relatives) ; Antibiotics, such as Hydroxyurea, Anthracyclines (e.g., doxorubicin agent, daunorubicin, epirubicin and other derivatives); Anthracenediones (e.g., Mitoxantrone and relatives); Streptomyces family (e.g., Bleomycin, Mitomycin C, Actinomycin, Plicamycin); and Ultraviolet light.

III. Kits for Use in Therapy

[0101] The present disclosure also provides kits for use in immunotherapy against cancer (e.g., melanoma, lung cancer, colorectal cancer, or renal-cell cancer), and/or treating or reducing the risk for cancer. Such kits can include one or more containers comprising a a synthetic oncolytic virus -containing pharmaceutical composition, e.g., any of those described herein.

[0102] In some instances, the kit can comprise instructions for use in accordance with any of the methods described herein. For example, the included instructions can comprise a description of administration of the a synthetic oncolytic virus-containing composition to treat, delay the onset, or alleviate a target disease as those described herein. The kit may further comprise a description of selecting an individual suitable for treatment based on identifying whether that individual has the target disease. In still other instances, the instructions comprise a description of administering the synthetic oncolytic virus -containing composition to an individual at risk of the target disease.

[0103] The instructions relating to the use of a synthetic oncolytic virus-containing composition generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

[0104] The label or package insert indicates that the composition is used for treating, delaying the onset and/or alleviating a disease or disorder associated with cancer, such as those described herein. Instructions may be provided for practicing any of the methods described herein.

[0105] The kits as described herein are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a synthetic oncolytic virus - containing composition such as those described herein.

[0106] Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container. In some instances, the invention provides articles of manufacture comprising contents of the kits described above.

IV. General techniques

[0107] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Molecular Cloning: A Laboratory Manual, second edition (Sambrook, et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M. J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R. I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J. P. Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, eds., 1993-8) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, eds., 1987); Current Protocols in Molecular

Biology (F. M. Ausubel, et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis, et al., eds., 1994); Current Protocols in Immunology (J. E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995).Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

[0108] Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present disclosure to its fullest extent. The following specific instances are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

EXAMPLES

Materials and Methods

Cell lines and Animals

[0109] Cell lines B16F10 (ATCC® CRL-6475™), HEK-blue-TLR2 (Invivogen), HEK-blue-TLR3 (Invivogen), HEK-blue-TLR7 (Invivogen), HEK-blue-TLR9 (Invivogen), Raw-Lucia ISG (Invivogen), and were cultured following vendor instructions (37°C, 5% CO_2). Female C57BL/6J (JAX Stock No. 000664) mice 6-8 weeks of age were purchased and maintained in the animal facility at the Massachusetts Institute of Technology (MIT). All animal studies and procedures were carried out following federal, state, and local guidelines under an IACUC-approved animal protocol by Committee of Animal Care at MIT.

Antibodies, Staining, and FACS Analysis

[0110] Antibodies against mouse Ly6c (HK1.4), CD11b (M1/70), CD11c (N418), F4/80 (BM8), MHC-II (M5/114.15.2), CD45 (30-F11), CD3 (17A2), CD4 (GK1.5), CD8 (53-6.7), NK1.1 (PK136), CD45.2 (104), CD24 (30-F1), XCR1 (ZET), CD64 (X54-5/7.1) were from Biolegend. Antibodies against mouse Ly6G (1A8), CD16/32 (2.4G2) and CD103 (M290) were from BD Biosciences. Antibodies against mouse Calreticulin (ab2907) was from Abcam. The live/dead dye (L34966) were from ThermoFisher.

[0111] The B16F10 melanoma tumor bearing mice were sacrificed and necropsied following federal, state, and local guidelines under an IACUC-approved animal protocol by Committee of Animal Care at MIT. Then the tumor draining lymph nodes were ground, and the tumors were sliced and digested by collagenase IV (1 mg/ml) for one hour for single cell suspensions. The single cells suspensions were filtered by 70 μm nylon constrainer and stained as described [10].

[0112] The stained samples were analyzed by FACS analyzer (LSR-II or LSR-II-Fortessa) from BD Biosciences. analyzed on a BD-LSRII Fortessa analyzer. All flow cytometry data were analyzed by FlowJo (Flowjo LLC) and the plots were prepared using GraphPad Prism.

Constructs, *In Vitro* Transcription, Capping/Methylating for Replicon RNA, and Neon Transfection

[0113] Backbone of mutant replicon constructs were from an *in vitro* evolution in a previous study (in revision). The IL12-MSA and IL12-MSA-Lumican were amplified from plasmids from Prof. Dane Wittrup Lab, and engineered in the subgenomic region of mutant replicon.

[0114] Replicon RNAs were *in vitro* transcribed (IVT) from the templates of linearized VEE-constructs above using the MEGAscript™ T7 Transcription Kit (ThermoFisher) following the manufacturer's instructions. Resulting replicon RNAs were capped and methylated using the ScriptCap™ m7G Capping System and ScriptCap™ 2'-O-Methyltransferase Kit (Cellscript) according to the manufacturer's instructions. RNA purity was assessed by gel electrophoresis.

[0115] *In vitro* transfections were carried out using 5 μg RNA for per 500,000 cells in 100 μl R buffer of NEON electroporation kit (ThermoFisher) at the conditions of 1200 voltage, 20 milliseconds, and 1 pulse.

Formulations of lipid nanoparticles and encapsulation of replicon RNA

[0116] For encapsulating 10 μg replicon RNA into DOTAP nanoparticle, a lipid mixture composed of 16.9375 μl DOTAP (Avanti, Cat#890890, 10 mg/ml), 15.965 μl DSPC (Avanti, Cat#850365, 3mg/ml), 18.7675 μl cholesterol (Sigma-Aldrich, Cat#C8667, 6 mg/ml), 13.6 μl DSPE-PEG2000 (Avanti Cat#880128, 2.5 mg/ml) in a molar ratio of 40:10:48:2 was

prepared in ethanol and evaporated under N_2 till one third of the total initial volume remained. Then 10 μg replicon RNA (1 mg/ml) in 11.8 μl 0.1 M citrate buffer (PH 6.0) was added with pipetting, followed by a second addition of an additional 22 μl 0.1 M citrate buffer (PH 6.0) with pipetting. The mixture was shaken for an hour and then dialyzed against PBS for another hour at 25°C in a 3,500 MWCO dialysis cassette.

[0117] Encapsulating replicon RNA into lipofectamine nanoparticle was following the instruction of Lipofectamine™ MessengerMAX™ Transfection Reagent (thermofisher.com/order/catalog/productALMRNA008).

[0118] For encapsulating 10 μg replicon RNA into TT3 nanoparticle, a lipid mixture composed of 10 μl TT3 (10 mg/ml) [11], 8.04 μl DOPE (Avanti, Cat# 850725, 10 mg/ml), 5.572 μl cholesterol (Sigma-Aldrich, Cat#C8667, 10 mg/ml), 3.452 μl C14-PEG2000 (Avanti Cat# 880150, 2 mg/ml) in a molar ratio of 20:30:40:0.75 was prepared in 10.437 μl ethanol. The mixture were added 4.167 μl citrate buffer (PH 3.0, 10 mM). Then 10 μg replicon RNA (1 mg/ml) in 31.667 μl citrate buffer (PH 3.0, 10 mM) was added with pipetting. The mixture was dialyzed against PBS for 80 minutes at 25°C in a 3,500 MWCO dialysis cassette.

[0119] The resulting replicon-loaded lipid nanoparticles were aliquoted in appropriate dosages for intratumoral injection (10 μg/mouse) and for transfection in vitro (5 μg/0.5 million cells in 500 μl media).

Annexin V/PI staining, ATP assay, and Elisa

[0120] Annexin V/PI staining follows the instruction of the kit from Biolegend (Cat#640932). Extracellular ATP was assayed by ENLITEN® ATP Assay System (Promega). HMGB1, CCL5, IFNα2, IL12, IFNγ were measured by ELISA kits from Chondrex (Cat#6010, HMGB1), R & D System (Cat#DY478, CCL5), Abcam (Cat#ab215409, IFNα2), and Biolegend (Cat#88-7121-88, IL12, Cat#88-7314-88, IFNγ) and followed their manuals.

RNA Extraction and Quantitative PCR Analysis

[0121] To quantify levels of RNA transcripts, total RNA was extracted from cells or tumors transfected with LNP-replicon RNA as indicated and reverse transcribed by a TaqMan™ Reverse Transcription Reagents Kit (ABI Catalog No. N8080234), followed by amplification with Sybr Green Master Mix (Roche) and specific primers for Stat1 (Cat#MP215434), Stat2 (Cat#MP215434), IRF9 (Cat#MP206708), IRF3 (Cat#MP206702), cGAS (Cat#MP214711) and detected by a Roche LightCycler 480. The Ct values were normalized with housekeeping gene mouse Actin B for comparison.

Example 1: Synthetic All in One LNP Replicon RNA for Cancer Immunotherapy

[0122] Therapeutic processes can be simplified and therapeutic efficacy can be amplified by synthetic multifunctional lipid nanoparticles (LNPs) encapsulating replicon RNA, with the lipid and RNA components each serving multiple roles: a lipid formulation that both promotes cellular uptake/cytosolic delivery of RNA while also directly triggering immunogenic cell death. In tandem, this LNP delivers self-amplifying replicon RNA that both encodes immunomodulatory therapeutic proteins and directly provides immunostimulation amplifying the subsequent immune response. Functionally, this synthetic LNP replicon RNA should induce local immunogenic cell death in tumor and also express immunomodulatory decently in transfected cells. Immunogenic cell death could enhance tumor infiltration by immune cells and provide a reservoir of tumor-specific antigens that could be cross presented to prime new T cells responses. LNP formulations containing the cationic lipid TT3 was identified as being especially relevant for these goals: 3 cationic lipid nanoparticles, each comprising a key cationic lipid- DOTAP, Lipofectamine (Lipo) or TT3, were compared. As the RNA cargo, an alphavirus replicon, derived from Venezuela Equine Encephalitis virus where the structural proteins were replaced by a cargo gene of interest inserted under the subgenomic promoter, was employed. When formulated with this self-amplifying replicon RNA (LNP-mtRep), the DOTAP, lipo, and TT3 lipid formulations formed particles with mean diameters of 97, 46, and 105 nm nanoparticles, with zeta potentials of +22.9, -6.7, and +4.3 mv, respectively (Fig. 1G-1H). The toxicity of the "empty" vs. RNA-loaded (mtRep) LNPs was assessed by incubating each formulation with B16F10 melanoma tumor cells in vitro. This assay revealed that viability significantly decreased for cells treated with TT3 LNPs, and mtRep synergized with TT3 to promote further tumor cell killing at 3-day post transfection (Fig. 1A). TT3 LNPs were more effective than DOTAP or Lipo in promoting tumor cell death. Notably, electroporation of replicon directly into tumor cells was relatively nontoxic, indicating that cell killing is promoted by the LNP delivery. To determine whether TT3 LNP-delivered replicons could drive cargo gene expression prior to cell death, we evaluated expression of the reporter gene GFP following LNP(mtRep) treatment. TT3-mtRep treatment led to ~35.4% of B16F10 cells to express GFP 12 hours post transfection, lower than electroporation (~90%), but significantly better than DOTAP-mtRep (~0.05%) or Lipo-mtRep (~7.6%) treatments (Fig. 1B). Consistent with Figure 1A, the cells transfected with TT3 nanoparticles showed a large population of Annexin V+/PI+ dead cells and mtRep again synergized to this cell death (Fig. 1C).

[0123] To determine if the cell death triggered by TT3(mtRep) is a type of immunogenic cell death (ICD), calreticulin

(CRT), which generally stays on endoplasmic reticulum (ER) and traffics to cell surface as an eat-me signal during ICD1, was measured. TT3 and mtRep synergized to promote the CRT trafficking to cell surfaces (Fig. 1D). Extracellular ATP activates the NLRP3 inflammasome2 and extracellular HMGB 1 mediates inflammation3 during ICD. TT3, TT3-mtRep, and TT3-deRep effectively induced ATP and HMGB1 release. (Fig. 1E and 1F). Taken it together, TT3-mtRep RNA is a promising oncolytic formulation that could induce immunogenic cell death while also leading to transient expression of cargo genes encoded by the replicon.

Example 2: Replicon RNA Triggers TLR3 Signaling and induces the ISGF3 Complex linked to Necrotic Cell Death

[0124] To determine the mechanisms underlying the synergy of cell death by replicon and TT3 LNP, DOTAP, Lipo, or TT3 nanoparticles with or without encapsulated mtRep encoding a reporter gene (or encapsulating a mutant "dead" replicon (deRep) that lacks functional gene expression) were transfected into reporter cells HEK-TLR2, HEK-TLR3, HEK-TLR7, or HEK-TLR9 (invivogen.com). Both Lipo and TT3 LNPs carrying mtRep or deRep activated TLR3 signaling, but none of the other TLRs tested were stimulated (Fig. 2A-D). When tested on Raw-Lucia-ISG reporter cells (invivogen.com), interferon stimulated genes were significantly induced by these same LNP formulations (Fig. 2E). These data suggest TLR3 recognizes replicon RNA and induces interferon responses in response to LNP-mediated delivery.

[0125] Since replicon RNA activates TLR3 signaling through TRIF for Type I interferon production[4] that leads to activation of the ISGF3 (Stat 1/Stat 2/IRF9) complex for necrotic cell death[5], we assayed mRNA transcript levels of ISGF3 complex components, Stat1, Stat2, and IRF9, as well as STING pathway genes, IRF3 and cGAS by qPCR. TT3 nanoparticles encapsulating mtRep or deRep increased ~6, ~16, and ~3 times the levels of Stat1, Stat2, IRF9 over untreated controls (Fig. 2F-H), respectively. In contrast, it had no effects on the transcription of IRF3 or cGAS (Fig. 2I-J). The DOTAP and Lipo formulations failed to induce the ISGF3 complex, likely because of low transfection efficiency of replicon RNA by these nanoparticles (Fig. 1B). These data suggested that mtRep and deRep likely activate TLR3 signaling and induce ISGF3 complex to promote necrotic cell death, a type of immunogenic cell death.

Example 3: TT3-mtRep Recruits Immune Cells and Regresses Established Tumors

[0126] Whether TT3-mtRep has effects on immunogenic cell death and expression of cargo genes *in vivo* was of interest. The absolute number of $Ly6c^{lo}$ $Ly6G^{+}$ granulocytic populations, which are associated with responses to necrotic cell death [6], was measured in tumors at 3-day post intratumoral injection of TT3 nanoparticles encapsulating wild type replicon RNA (wtRep), with another mutant replicon RNA (mt2Rep), and the mutant replicon (mtRep) used above. Unexpectedly, the mtRep showed significantly greater (~2 fold higher) recruitment of this granulocytic population to tumors (Figure 3A), and better expression of the reporter gene mCherry encoded by the subgenomic promoter (Fig. 3B). Thus, TT3-mtRep induces immunogenic cell death with decent cargo gene expression. In vivo studies using TT3-mtRep were carried out subsequently.

[0127] The expression level of Stat1/Stat2/IRF9 as well as STING signaling genes IRF3 and cGAS in vivo following intratumoral LNP delivery of replicons was measured. Consistent with the Fig. 2F-J *in vitro,* tumors injected with TT3-mtRep expressed significantly higher Stat1/Stat2/IRF9 (ISGF3 complex), but with comparable levels of IRF3 and cGAS (Fig. 3C), suggesting administration of TT3-mtRep also initiated necrotic cell death *in vivo.*

[0128] To better understand the effects of TT3-mtRep to immune composition in tumor, immune cells such as granulocytes ($CD45^{+}$ $CD11b^{+}$ $Ly6c^{lo}$ $Ly6G^{+}$), M-MDSC ($CD45^{+}$ $CD11b^{+}$ $Ly6c^{hi}$ $Ly6G^{+}$), Monocytes ($D45^{+}$ $CD11b^{+}$ $Ly6c^{lo}$ $Ly6G^{-}$), Macrophages ($CD45^{+}$ $CD11b^{+}$ $Ly6c^{-}$ Ly6G F4/80^{+}), CD4 T ($CD45^{-}$ $CD3\varepsilon^{+}$ $CD4^{+}$), CD8 T ($CD45^{+}$ $CD3\varepsilon^{+}$ $CD8^{+}$), NK ($CD45^{+}$ $CD3\varepsilon^{-}$ $NK1.1^{+}$), NKT ($CD45^{+}$ $CD3\varepsilon^{+}NK1.1^{+}$), conventional DC1 (cDC1, $CD45^{+}$ $CD11c^{+}$ MHC-II^{+} $CD24^{+}$ $CD64^{-}$ $CD103^{+}$ $CD11b^{-}$ $XCR1^{hi}$), and conventional DC2 (cDC2, $CD45^{+}$ $CD11c^{+}$ MHC-II^{+} $CD24^{+}$ $CD64^{-}$ $CD103^{+}$ $CD11b^{+}$ $XCR1^{lo}$), ware mapped and quantified in the tumors at 1 day (Fig. 3D) and 3 days (Fig. 3E) post one injection, and at 1 day (Fig. 3F) post three sequential injections of LNP-replicon RNA. In this dynamic analysis of immune compositions in tumor, the granulocytes and the cDC1 were quickly recruited and decreased at 1 day post injection of replicon RNA, respectively (Figure 3C), suggesting granulocytes might be early event and cDC1 likely start trafficking to tumor draining lymph node (TDLN) in response to LNP-replicon RNA. At 3 days post injection of replicon RNA, CD4 T, CD8 T, NK, and NKT cells were also recruited (Figure 3D). When administering 3 sequential injections of LNP-replicon RNA, monocytes increased and macrophages decreased, but less significant changes were observed in lymphoid cells, such as CD4 T, NK, and NKT cells (Figure 3E). As effects of these ~3 sequential injections, there was ~10% more cell death (Figure 3F), ~4 times higher tumor infiltrating immune cells (Figure 3G), and a 2-fold reduction in tumor weight (Figure 3H) in the samples of TT3-mtRep group, resulting in significant regression of tumor growth (Figure 3I). Consistent with the NK, and NKT cells come up along with the treatments of TT3-mtRep, CCL5 expression was significantly induced (Figure 3I), which is mainly secreted by NK [7] and activated CD8 T cells (rstats.immgen.org/Skyline/skyline.html). These data indicate TT3-mtRep induced immunogenic cell death in tumors with expression of cargo genes as observed in Fig. 1 and Fig. 2 *in vitro.* Most importantly, TT3-

mtRep significantly modulated the tumor microenvironment, and led to tumor recruitment of CD8 T cells and NK cells.

Example 4: TT3-mtRep Encoding with IL12-MSA or ILl2-MSA-Lumican Effectively Modulates Tumor Microenvironments and Immune Composition

[0129] While intratumoral injection of TT3(mtRep) encoding reporter genes alone led to tumor growth delay, encoding immunomodulatory proteins in the replicon could lead to further immunostimulation and enhancements of anti-tumor immunity. An attractive candidate is IL-12, which could polarize CD4 T helpers to Th1 and enhance cytotoxicity effects of NK and CD8 T cells [8]. One of the subunit of IL12, also named as IL12b or IL12p40, is mainly secreted from antigen presentation cells ($CD8^+$ DCs) (rstats.immgen.org/Skyline/skyline.html), which could be functionally activated by Type I interferon [9]. However, the interferon $\alpha 2$ (IFN$\alpha 2$), a main form of Type I interferon, is low either in the serum (Fig. 4A) or in the tumors (Fig. 4B) at day 1 and day 3 following TT3-mtRep injections, suggesting IL-12 secreted by DCs may still be low in the tumor microenvironment (TME). Thus, IL12-MSA or IL12-MSA-Lumican that fused IL12α (P30), IL12β (P40) with murine serum albumin (MSA), or with MSA and Lumican was engineered. Lumican is an endogenous collagen-binding protein, which we reasoned would promote retention of expressed IL-12 in the TME to enhance efficacy and safety of the replicon therapy. The replicon constructs were transfected in B16F10 cells in vitro by NEON transfection and we validated IL12 secretion in these transfected cells by ELISA (Fig. 4C).

[0130] Consistently, tumors injected with TT3-mtRep encoding IL12-MSA (TT3-mtRep-IL12-MSA) or IL12-MSA-Lumican (TT3-mtRep-IL12-MSA-Lumican) expressed high levels of IL12 in vivo, reaching 40 ng/mg in tumors (Fig. 4D). Comparing immune composition in tumor at day 1 post injections, granulocytes was 1.5-3 times increased in the tumors treated with TT3-mtRep expressing IL12-MSA and IL12-MSA-Lumican, in contrast to the group treated with TT3-mtRep encoding with reporter gene mCherry (Fig. 4E). At day 3, IL12-MSA and IL12-MSA-Lumican groups recruited ~2-3 times higher granulocytes, CD8 T cells, and cDC2 cells to tumors compared to replicons encoding an irrelevant reporter gene (Fig. 4F). Interestingly, tumors treated with TT3-mtRep encoding reporter genes, IL12-MSA, or IL12-MSA-Lumican all showed decreases of cDC1 cells in comparison to the untreated group (Fig. 4E-4F). To determine if cDC1 were trafficking to the TDLN, we numerated the cDC1 cells in it and showed the cDC1 cells in lymph nodes significantly increased after TT3-mtRep treatment, no matter what cargo gene was encoded (Fig. 4G). Increases of cDC1 cells continued in the IL12-MSA group (Fig. 4G).

Example 5: Immunomodulatory IL12 and ICD Induced by TT3-mtRep Effectively Eradicate B16F10 Tumor

[0131] As administrating IL12 protein has severe side effects in clinic studies, we measured the body weight changes of tumor bearing mice treated with replicon-encoded IL-12. The mtRep-IL12-MSA group had ~ 5% body loss that lasted a week. In contrast, mtRep and mtRep-IL12-MSA-Lumican groups had only ~2% body loss and recovered in a few days (Fig. 5A). Consistently, we failed to measure IL-6 or TNFα in serum at day 1 or day 3 post injection of mtRep-IL12-MSA or mtRep-IL12-MSA-Lumican (data not shown). But, we did observe significant increases of IFN-γ in serum in the group of mtRep-IL12-MSA (Fig. 5B), consistent with the body loss in Fig. 5A. Interestingly, the replicons expressing IL12-MSA or IL12-MSA-Lumican showed dramatic tumor regression, even to large established tumors with sizes at treatment of 50 mm^2 (Fig. 5C). The tumor bearing mice treated with TT3-mtRep encoding with IL12-MSA and IL12-MSA-Lumican were 60% and 80% tumor free at 75 days post B16F10 injection, respectively. To determine if these cured mice had elicited a systemic immune response that could prevent B16F10 tumor recurrence, these mice were challenged with 0.1 million B16F10 cells in opposite flank. As expected, all of the cured mice rejected the B16F10 tumor in comparison of the naive mice that quickly development of B16F10 tumor. These data indicate that synergy of immunomodulatory IL12 and ICD (immunogenic cell death) could effectively eradicate B16F10 tumor and induce systemic immune responses to prevent the recurrence of B16F10 tumor.

References:

[0132]

1 Yun, Y. R. et al. Fibroblast growth factors: biology, function, and application for tissue regeneration. J Tissue Eng 2010, 218142, doi:10.4061/2010/218142 (2010).

2 Zha, Q. B. et al. ATP-Induced Inflammasome Activation and Pyroptosis Is Regulated by AMP-Activated Protein Kinase in Macrophages. Front Immunol 7, 597, doi:10.3389/fimmu.2016.00597 (2016).

3 Magna, M. & Pisetsky, D. S. The role of HMGB1 in the pathogenesis of inflammatory and autoimmune diseases. Mol Med 20, 138-146, doi:10.2119/molmed.2013.00164 (2014).

4 Kawai, T. & Akira, S. TLR signaling. Cell Death Differ 13, 816-825, doi:10.1038/sj.cdd.4401850 (2006).

5 McComb, S. et al. Type-I interferon signaling through ISGF3 complex is required for sustained Rip3 activation and

necroptosis in macrophages. Proc Natl Acad Sci U S A 111, E3206-3213, doi:10.1073/pnas.1407068111 (2014).
6 Haverkamp, J. M. et al. Myeloid-derived suppressor activity is mediated by monocytic lineages maintained by continuous inhibition of extrinsic and intrinsic death pathways. Immunity 41, 947-959, doi:10.1016/j.immuni.2014.10.020 (2014).
7 Bottcher, J. P. et al. NK Cells Stimulate Recruitment of cDC1 into the Tumor Microenvironment Promoting Cancer Immune Control. Cell 172, 1022-1037 e1014, doi:10.1016/j.cell.2018.01.004 (2018).
8 Lasek, W., Zagozdzon, R. & Jakobisiak, M. Interleukin 12: still a promising candidate for tumor immunotherapy? Cancer Immunol Immunother 63, 419-435, doi:10.1007/s00262-014-1523-1 (2014).
9 Montoya, M. et al. Type I interferons produced by dendritic cells promote their phenotypic and functional activation. Blood 99, 3263-3271, doi:DOI10.1182/blood.V99.9.3263 (2002).
10 Li, Y. et al. Persistent Antigen and Prolonged AKT-mTORC1 Activation Underlie Memory CD8 T Cell Impairment in the Absence of CD4 T Cells. J Immunal 195, 1591-1598, doi:10.4049/jimmunol.1500451 (2015).
11 Li, B. et al. An Orthogonal Array Optimization of Lipid-like Nanoparticles for mRNA Delivery in Vivo. Nano Lett 15, 8099-8107, doi:10.1021/acs.nanolett.5b03528 (2015).

OTHER INSTANCES

[0133] All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

EQUIVALENTS

[0134] All definitions, as defined and used herein, should be understood to control over dictionary definitions, and/or ordinary meanings of the defined terms.

[0135] The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one." The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, i.e., "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[0136] As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

[0137] As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

[0138] It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

Claims

1. A synthetic oncolytic virus, comprising:

   (i) a lipid nanoparticle comprising one or more types of lipid, wherein the one or more types of lipid comprises N1,N3,N5-tris(3-(didodecylamino)propyl)benzene-1,3,5-tricarboxamide (TT3); and
   (ii) a self-amplifying viral replicon RNA comprising a sequence that encodes an interleukin (IL)-12 molecule;

   and
   wherein the IL-12 molecule is expressed by the self-amplifying viral replicon RNA.

2. The synthetic oncolytic virus of claim 1, wherein the lipid nanoparticle further comprises 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, and C14-PEG2000.

3. The synthetic oncolytic virus of any one of claims 1 or 2, wherein the self-amplifying viral replicon RNA is derived from an alphavirus or a hepatitis C virus.

4. The synthetic oncolytic virus of claim 3, wherein the alphavirus is Venezuela Equine Encephalitis virus, Semliki Forest virus, or Sindbis virus.

5. The synthetic oncolytic virus of any one of claims 1-4, wherein the sequence that encodes the IL-12 molecule is located under a subgenomic promoter in a subgenomic region of the self-amplifying viral replicon RNA.

6. The synthetic oncolytic virus of any one of claims 1-5, wherein the self-amplifying viral replicon RNA comprises a nucleotide sequence that has at least 90% sequence identity to the sequence set forth in SEQ ID NO: 1.

7. The synthetic oncolytic virus of claim 6, wherein the self-amplifying viral replicon RNA comprises a point mutations of G3936C and/or A4758G of SEQ ID NO: 1.

8. The synthetic oncolytic virus of any one of claims 1-7, wherein the IL-12 molecule is selected from the group consisting of IL-12, an IL-12 subunit, or a mutant IL-12 molecule that retains the immunomodulatory function.

9. The synthetic oncolytic virus of claim 8, wherein the IL-12 molecule comprises IL-12α and/or IL-12β subunits.

10. The synthetic oncolytic virus of any one of claims 1-9, wherein the lipid nanoparticle has a diameter of about 100-120 nm and/or has a zeta potential of about 3-6 mv.

11. The synthetic oncolytic virus of any one of claims 1-10, wherein the lipid nanoparticle is capable of triggering immunogenic cell death.

12. A pharmaceutical composition, comprising the synthetic oncolytic virus of any one of claims 1-11 and a pharmaceutically acceptable carrier.

13. The synthetic oncolytic virus of any one of claims 1-11 or the pharmaceutical composition of claim 12 for use in treating a cancer in a subject.

14. The synthetic oncolytic virus or the pharmaceutical composition for the use of claim 13, wherein the subject is a human patient having or suspected of having a cancer, wherein the cancer is melanoma, breast cancer, colon cancer, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, gastric cancer, squamous cell head and neck cancer, renal-cell cancer, urothelial carcinoma, or Hodgkin's lymphoma.

Patentansprüche

1. Synthetisches onkolytisches Virus, umfassend:

   (i) ein Lipid-Nanopartikel, das eine oder mehrere Arten von Lipid umfasst, wobei die eine oder mehreren Arten von Lipid N1,N3,N5-tris(3-(didodecylamino)propyl)benzol-1,3,5-tricarboxamid(TT3) umfassen; und
   (ii) eine selbstverstärkende Virus-Replicon-RNA, die eine Sequenz umfasst, die für ein Interleukin (IL)-12-Molekül kodiert;

   und
   wobei das IL-12-Molekül durch die selbstverstärkende Virus-Replicon-RNA exprimiert wird.

2. Synthetisches onkolytisches Virus nach Anspruch 1, wobei der Lipid-Nanopartikel ferner 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamin (DOPE), Cholesterin und C14-PEG2000 umfasst.

3. Synthetisches onkolytisches Virus nach einem der Ansprüche 1 oder 2, wobei die selbstverstärkende Virus-Replicon-RNA von einem Alphavirus oder einem Hepatitis-C-Virus abgeleitet ist.

4. Synthetisches onkolytisches Virus nach Anspruch 3, wobei das Alphavirus das Venezolanische Pferdeenzephalomyelitis-Virus, das Semliki-Forest-Virus oder das Sindbis-Virus ist.

5. Synthetisches onkolytisches Virus nach einem der Ansprüche 1-4, wobei sich die Sequenz, die für das IL-12-Molekül kodiert, unter einem subgenomischen Promotor in einer subgenomischen Region der selbstverstärkende Virus-Replicon-RNA befindet.

6. Synthetisches onkolytisches Virus nach einem der Ansprüche 1-5, wobei die selbstverstärkende Virusreplicon-RNA eine Nukleotidsequenz umfasst, die mindestens 90 % Sequenzidentität mit der Sequenz in SEQ ID NO: 1 aufweist.

7. Synthetisches onkolytisches Virus nach Anspruch 6, wobei die selbstverstärkende Virusreplicon-RNA eine Punktmutation von G3936C und/oder A4758G von SEQ ID NO: 1 umfasst.

8. Synthetisches onkolytisches Virus nach einem der Ansprüche 1-7, wobei das IL-12-Molekül ausgewählt ist aus der Gruppe bestehend aus IL-12, einer IL-12-Untereinheit oder einem mutierten IL-12-Molekül, das die immunmodulatorische Funktion beibehält.

9. Synthetisches onkolytisches Virus nach Anspruch 8, wobei das IL-12-Molekül IL-12α und/oder IL-12β-Untereinheiten umfasst.

10. Synthetisches onkolytisches Virus nach einem der Ansprüche 1-9, wobei das Lipid-Nanopartikel einen Durchmesser von etwa 100-120 nm aufweist und/oder ein Zeta-Potenzial von etwa 3-6 mv aufweist.

11. Synthetisches onkolytisches Virus nach einem der Ansprüche 1-10, wobei das Lipid-Nanopartikel in der Lage ist, den immunogenen Zelltod auszulösen.

12. Pharmazeutische Zusammensetzung, umfassend das synthetische onkolytische Virus nach einem der Ansprüche 1-11 und einen pharmazeutisch unbedenklichen Trägerstoff.

13. Synthetisches onkolytisches Virus nach einem der Ansprüche 1-11 oder pharmazeutische Zusammensetzung nach Anspruch 12 zur Verwendung bei der Behandlung eines Krebses bei einem Subjekt.

14. Synthetisches onkolytisches Virus oder pharmazeutische Zusammensetzung zur Verwendung nach Anspruch 13, wobei der Patient ein menschlicher Patient ist, der einen Krebs hat oder bei dem ein Krebs vermutet wird, wobei der Krebs Melanom, Brustkrebs, Kolonkrebs, Plattenepithelkarzinom, kleinzelliges Lungenkarzinom, nicht-kleinzelliges Lungenkarzinom, Adenokarzinom der Lunge, Plattenepithelkarzinom der Lunge, Peritonealkarzinose, Leberzellkarzinom, Magen-Darm-Krebs, Bauchspeicheldrüsenkrebs, Glioblastom, Gebärmutterhalskrebs, Eierstockkrebs, Leberkrebs, Blasenkrebs, Hepatom, Kolorektalkrebs, Endometrium- oder Gebärmutterkarzinom, Speicheldrüsenkarzinom, Nierenkrebs, Prostatakkrebs, Vulvakrebs, Schilddrüsenkrebs, Leberkarzinom, Magenkrebs, Plattenepithelkarzinom des Kopfes und Halses, Nierenzellkarzinom, Urothelkarzinom oder Hodgkin-Lymphom ist.

Revendications

1. Virus oncolytique synthétique, comprenant :

   (i) une nanoparticule lipidique comprenant un ou plusieurs types de lipides, ledit ou lesdits types de lipides comprennent le N1,N3,N5-tris(3-(didodécylamino)propyl)benzène-1,3,5-tricarboxamide (TT3) ; et
   (ii) un ARN réplicon viral auto-amplifiant comprenant une séquence codant pour une molécule d'interleukine (IL)-12 ;

   et
   dans lequel la molécule d'IL-12 est exprimée par l'ARN réplicon viral auto-amplifiant.

2. Virus oncolytique synthétique de la revendication 1, dans lequel la nanoparticule lipidique comprend en outre de la 1,2-dioléoyl-sn-glycéro-3-phosphoéthanolamine (DOPE), du cholestérol et du C14-PEG2000.

3. Virus oncolytique synthétique de l'une quelconque des revendications 1 ou 2, dans lequel l'ARN réplicon viral auto-amplifiant est dérivé d'un alphavirus ou d'un virus de l'hépatite C.

4. Virus oncolytique synthétique de la revendication 3, dans lequel l'alphavirus est le virus de l'encéphalite équine du Venezuela, le virus de la forêt de Semliki ou le virus Sindbis.

5. Virus oncolytique synthétique de l'une quelconque des revendications 1 à 4, dans lequel la séquence codant pour la molécule d'IL-12 est située sous un promoteur sous-génomique dans une région sous-génomique de l'ARN réplicon viral auto-amplifiant.

6. Virus oncolytique synthétique de l'une quelconque des revendications 1 à 5, dans lequel l'ARN réplicon viral auto-amplifiant comprend une séquence nucléotidique qui présente une identité de séquence d'au moins 90 % avec la séquence définie dans SEQ ID N° : 1.

7. Virus oncolytique synthétique de la revendication 6, dans lequel l'ARN réplicon viral auto-amplifiant comprend des mutations ponctuelles de G3936C et/ou A4758G de SEQ ID N° : 1.

8. Virus oncolytique synthétique de l'une quelconque des revendications 1 à 7, dans lequel la molécule d'IL-12 est choisie dans le groupe constitué par l'IL-12, une sous-unité d'IL-12 ou une molécule d'IL-12 mutante conservant la fonction immunomodulatrice.

9. Virus oncolytique synthétique de la revendication 8, dans lequel la molécule d'IL-12 comprend des sous-unités d'IL-12α et/ou d'IL-12β.

10. Virus oncolytique synthétique de l'une quelconque des revendications 1 à 9, dans lequel la nanoparticule lipidique possède un diamètre d'environ 100 à 120 nm et/ou présente un potentiel zêta d'environ 3 à 6 mV.

11. Virus oncolytique synthétique de l'une quelconque des revendications 1 à 10, dans lequel la nanoparticule lipidique est capable de déclencher la mort cellulaire immunogène.

12. Composition pharmaceutique comprenant le virus oncolytique synthétique de l'une quelconque des revendications 1 à 11 et un support acceptable pharmaceutiquement.

13. Virus oncolytique synthétique de l'une quelconque des revendications 1 à 11 ou composition pharmaceutique de la revendication 12 destiné(e) à être utilisé(e) dans le traitement d'un cancer chez un sujet.

14. Virus oncolytique synthétique ou composition pharmaceutique destiné(e) à être utilisé(e) selon la revendication 13, ledit sujet étant un patient humain ayant ou soupçonné d'avoir un cancer, ledit cancer étant le mélanome, le cancer du sein, le cancer du côlon, le cancer épidermoïde, le cancer du poumon à petites cellules, le cancer du poumon non à petites cellules, l'adénocarcinome du poumon, le carcinome épidermoïde du poumon, le cancer du péritoine, le cancer hépatocellulaire, le cancer gastro-intestinal, le cancer du pancréas, le glioblastome, le cancer du col de l'utérus, le cancer de l'ovaire, le cancer du foie, le cancer de la vessie, l'hépatome, le cancer colorectal, le carcinome endométrial ou utérin, le carcinome des glandes salivaires, le cancer du rein, le cancer de la prostate, le cancer de la

vulve, le cancer de la thyroïde, le carcinome hépatique, le cancer de l'estomac, le cancer épidermoïde de la tête et du cou, le cancer à cellules rénales, le carcinome urothélial ou le lymphome de Hodgkin.

FIG. 1A

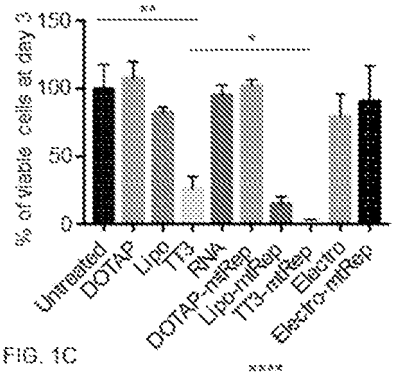


FIG. 1B

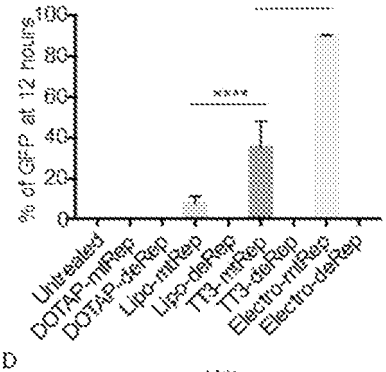


FIG. 1C

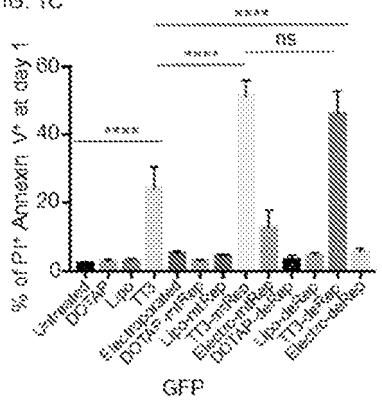


FIG. 1D

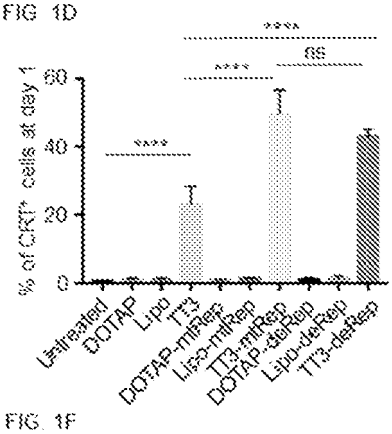


FIG. 1E

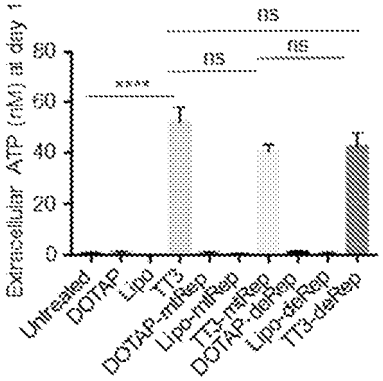


FIG. 1F

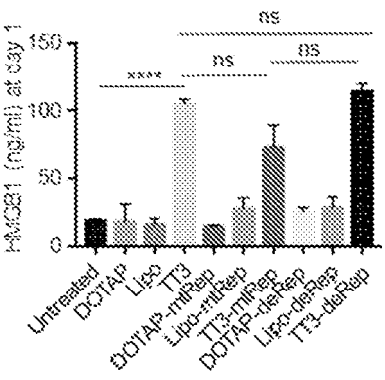


FIG. 1G

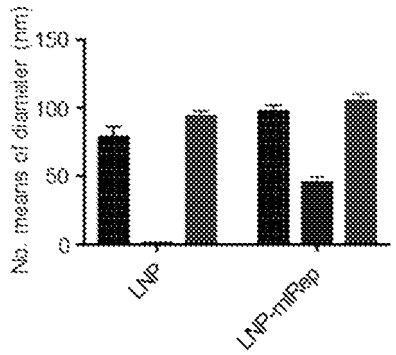


FIG. 1H

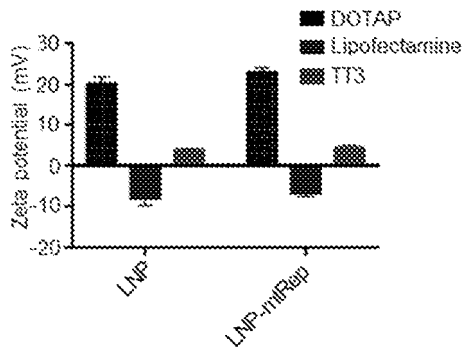


FIG. 2A

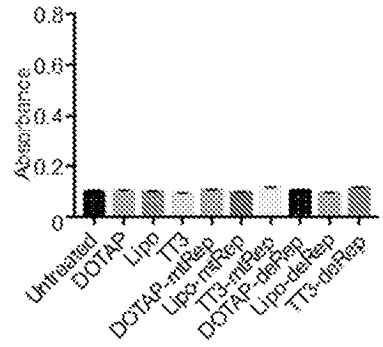


FIG. 2B

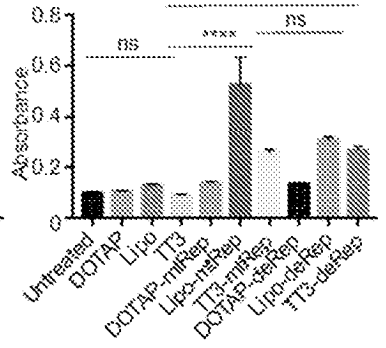


FIG. 2C

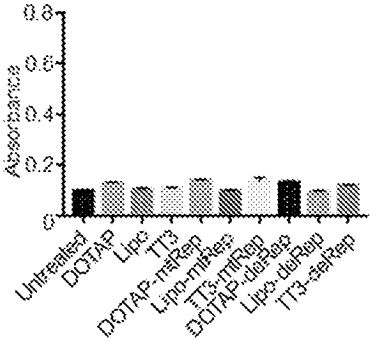


FIG. 2D

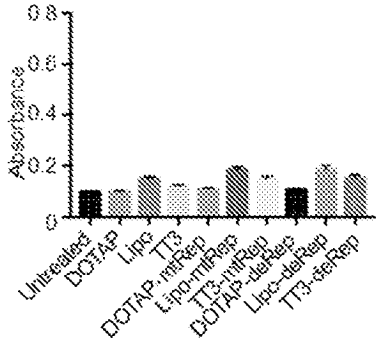


FIG. 2E

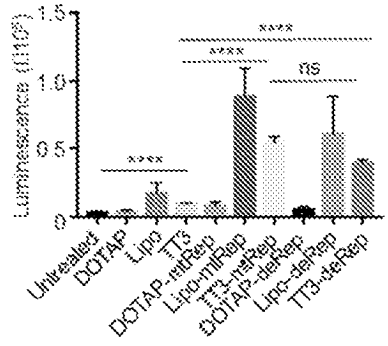


FIG. 2F

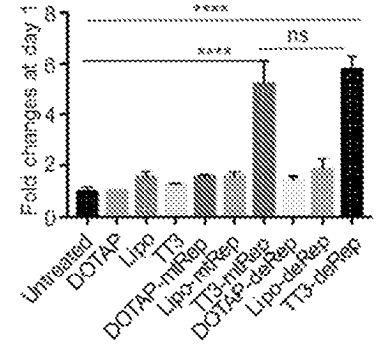


FIG. 2G

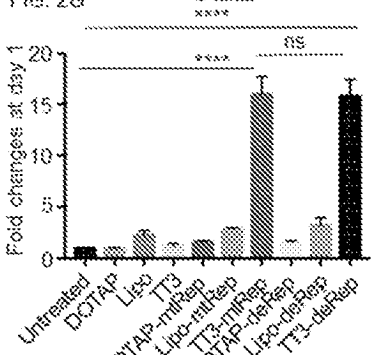


FIG. 2H

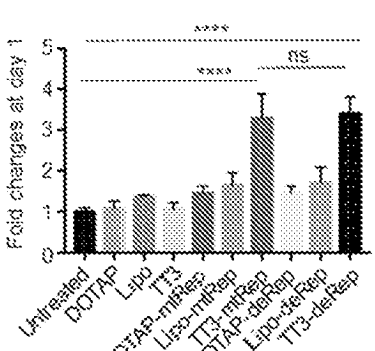


FIG. 2I

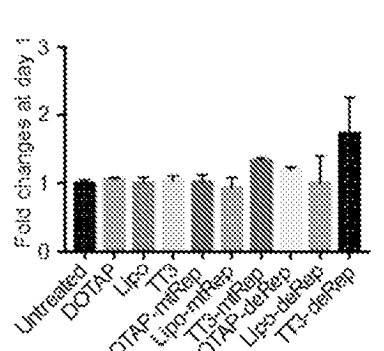


FIG. 2J

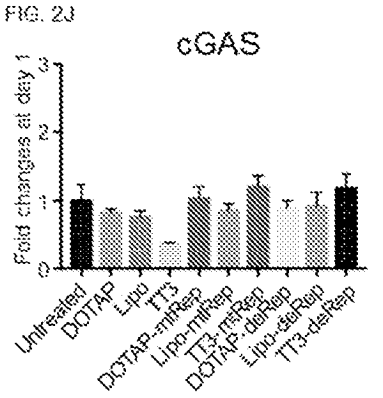


FIG. 3A

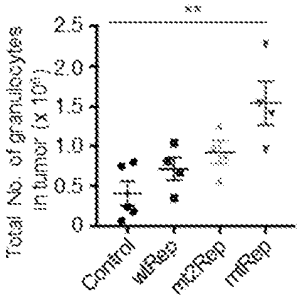


FIG. 3B

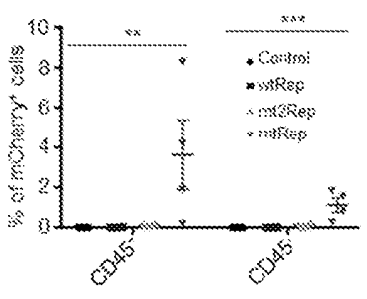


FIG. 3C

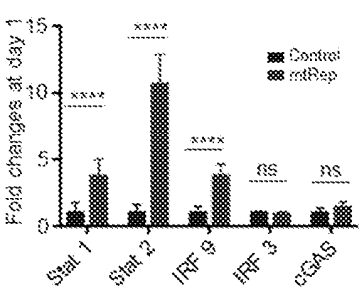


FIG. 3D

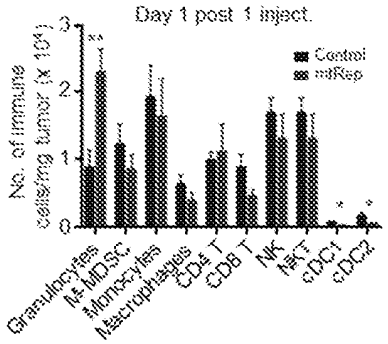


FIG. 3E

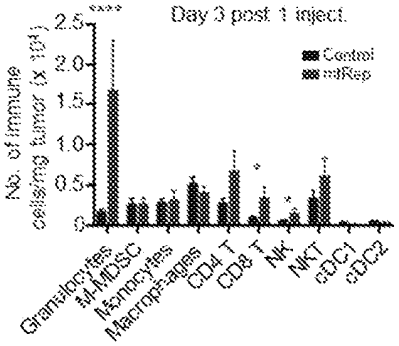


FIG. 3F

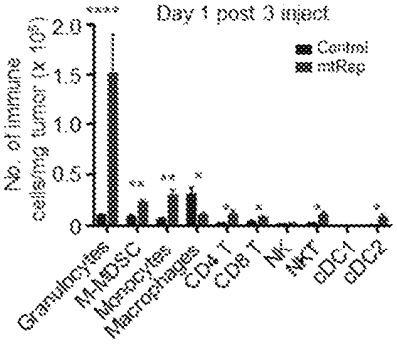


FIG. 3G

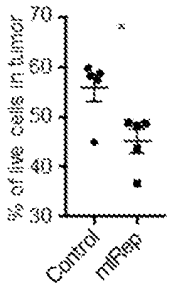


FIG. 3H



FIG. 3I

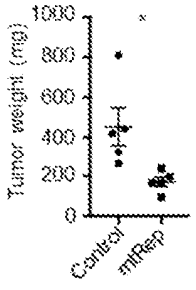


FIG. 3J

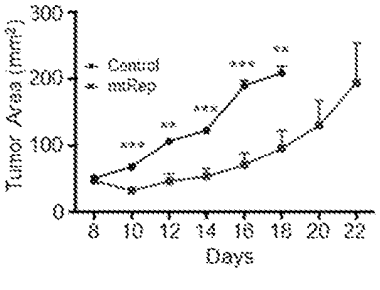


FIG. 3K



FIG. 4A

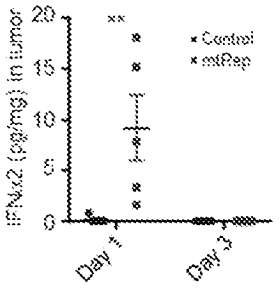


FIG. 4B

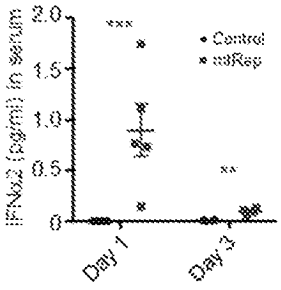


FIG. 4C

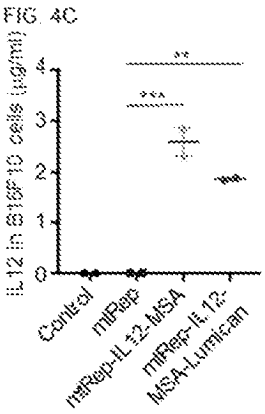


FIG. 4D

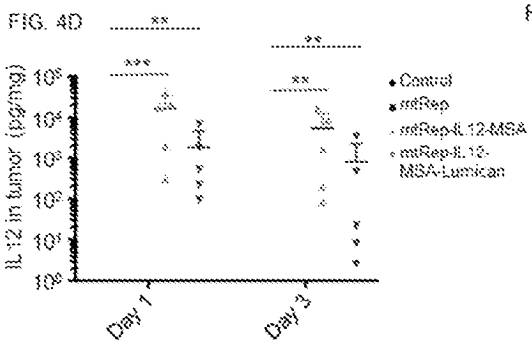


FIG. 4E

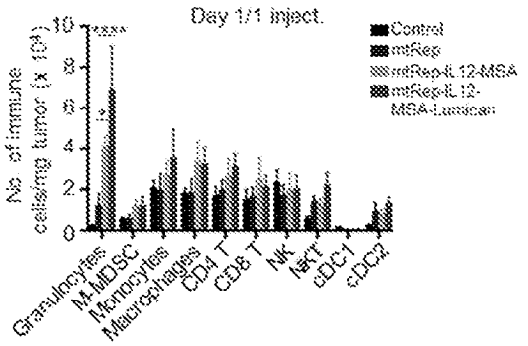


FIG. 4F

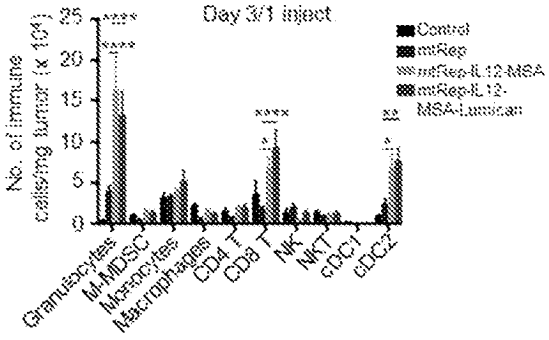


FIG. 4G

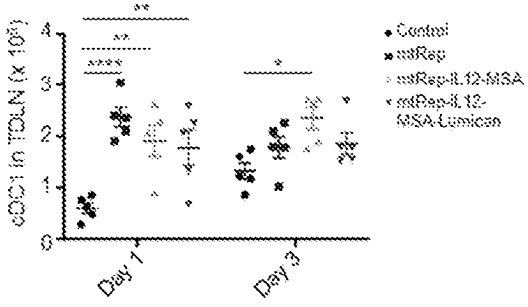


FIG. 5A

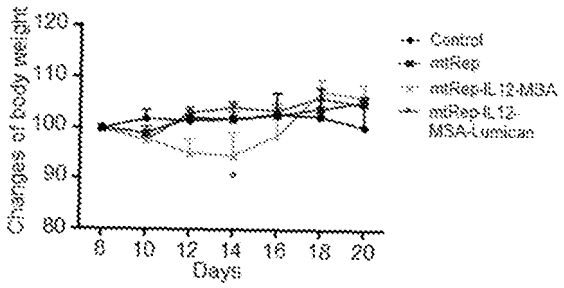


FIG. 5B

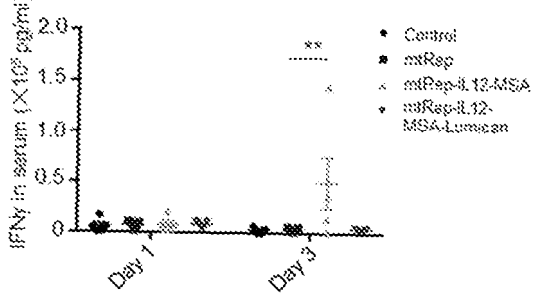


FIG. 5C

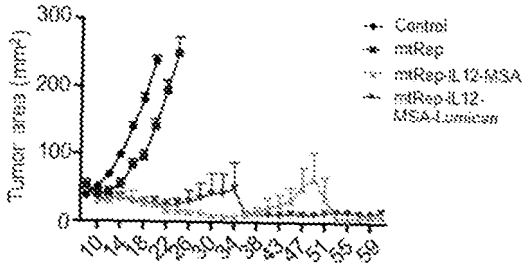


FIG. 5D

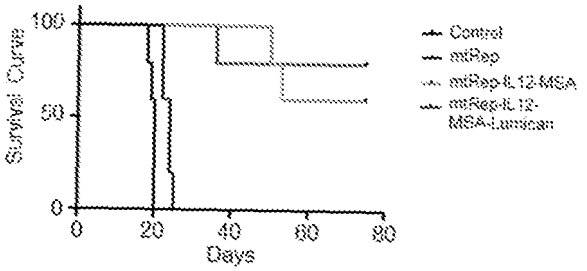
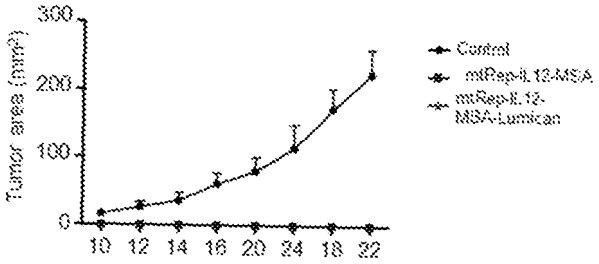


FIG. 5E



REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 62815611 **[0001]**
- US 4485045 A **[0072]**
- US 4544545 A **[0072]**
- US 5013556 A **[0072]**
- US 3773919 A **[0073]**

Non-patent literature cited in the description

- **LUNDSTROM K et al.** *Vaccines*, 2016, vol. 4 (4), 39 **[0003]**
- **LAI, I et al.** *Journal for ImmunoTherapy of Cancer*, 2018, vol. 6 (1:125), 1-11 **[0003]**
- **EPSTEIN et al.** *Proc. Natl. Acad. Sci. USA*, 1985, vol. 82, 3688 **[0072]**
- **HWANG et al.** *Proc. Natl. Acad. Sci. USA*, 1980, vol. 77, 4030 **[0072]**
- **SAMBROOK et al.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Press, 1989 **[0107]**
- Oligonucleotide Synthesis. 1984 **[0107]**
- Methods in Molecular Biology. Humana Press **[0107]**
- Cell Biology: A Laboratory Notebook. Academic Press, 1998 **[0107]**
- Animal Cell Culture. 1987 **[0107]**
- **J. P. MATHER** ; **P. E. ROBERTS**. Introduction to Cell and Tissue Culture. Plenum Press, 1998 **[0107]**
- Cell and Tissue Culture: Laboratory Procedures. J. Wiley and Sons, 1993 **[0107]**
- Methods in Enzymology. Academic Press, Inc. **[0107]**
- Handbook of Experimental Immunology **[0107]**
- Gene Transfer Vectors for Mammalian Cells. 1987 **[0107]**
- Current Protocols in Molecular Biology. 1987 **[0107]**
- PCR: The Polymerase Chain Reaction. 1994 **[0107]**
- Current Protocols in Immunology. 1991 **[0107]**
- Short Protocols in Molecular Biology. Wiley and Sons, 1999 **[0107]**
- **C. A. JANEWAY** ; **P. TRAVERS**. Immunobiology. 1997 **[0107]**
- **P. FINCH**. Antibodies. 1997 **[0107]**
- Antibodies: a practical approach. IRL Press, 1988 **[0107]**
- Monoclonal antibodies: a practical approach. Oxford University Press, 2000 **[0107]**
- **E. HARLOW** ; **D. LANE**. Using antibodies: a laboratory manual. Cold Spring Harbor Laboratory Press, 1999 **[0107]**
- The Antibodies. Harwood Academic Publishers, 1995 **[0107]**
- **YUN, Y. R. et al.** Fibroblast growth factors: biology, function, and application for tissue regeneration. *J Tissue Eng*, 2010, vol. 2010, 218142 **[0132]**
- **ZHA, Q. B. et al.** ATP-Induced Inflammasome Activation and Pyroptosis Is Regulated by AMP-Activated Protein Kinase in Macrophages. *Front Immunol*, 2016, vol. 7, 597 **[0132]**
- **MAGNA, M.** ; **PISETSKY, D. S.** The role of HMGB1 in the pathogenesis of inflammatory and autoimmune diseases. *Mol Med*, 2014, vol. 20, 138-146 **[0132]**
- **KAWAI, T.** ; **AKIRA, S.** TLR signaling. *Cell Death Differ*, 2006, vol. 13, 816-825 **[0132]**
- **MCCOMB, S. et al.** Type-I interferon signaling through ISGF3 complex is required for sustained Rip3 activation and necroptosis in macrophages. *Proc Natl Acad Sci U S A*, 2014, vol. 111, E3206-3213 **[0132]**
- **HAVERKAMP, J. M. et al.** Myeloid-derived suppressor activity is mediated by monocytic lineages maintained by continuous inhibition of extrinsic and intrinsic death pathways. *Immunity*, 2014, vol. 41, 947-959 **[0132]**
- **BOTTCHER, J. P. et al.** NK Cells Stimulate Recruitment of cDC1 into the Tumor Microenvironment Promoting Cancer Immune Control. *Cell*, 2018, vol. 172, 1022-1037, e1014 **[0132]**
- **LASEK, W.** ; **ZAGOZDZON, R.** ; **JAKOBISIAK, M.** Interleukin 12: still a promising candidate for tumor immunotherapy?. *Cancer Immunol Immunother*, 2014, vol. 63, 419-435 **[0132]**
- **MONTOYA, M. et al.** Type I interferons produced by dendritic cells promote their phenotypic and functional activation. *Blood*, 2002, vol. 99, 3263-3271 **[0132]**
- **LI, Y. et al.** Persistent Antigen and Prolonged AKT-mTORC1 Activation Underlie Memory CD8 T Cell Impairment in the Absence of CD4 T Cells. *J Immunal*, 2015, vol. 195, 1591-1598 **[0132]**
- **LI, B. et al.** An Orthogonal Array Optimization of Lipid-like Nanoparticles for mRNA Delivery in Vivo. *Nano Lett*, 2015, vol. 15, 8099-8107 **[0132]**