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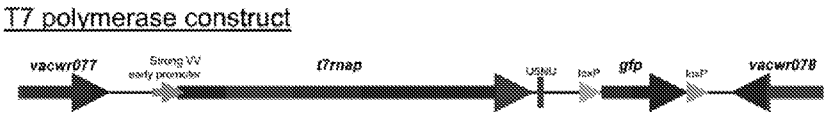
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FIG. 8



(57) Abstract: This disclosure provides compositions comprising a modified oncolytic virus that can contain modifications in the viral genome and exogenous nucleic acids coding for proteins. The viral compositions and methods provided herein can be utilized for the treatment of cancer. One embodiment provides a virus that produces from its genome a non-replicating retrovirus, wherein the virus is an oncolytic virus.

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PRODUCER VIRUSES FOR GENERATION OF RETROVIRUSES *IN SITU*

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/916,095 filed Oct 16, 2019, which is incorporated by reference herein in its entirety.

INCORPORATION BY REFERENCE

[0002] All publications, patents, patent applications, and NCBI accession numbers mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference, and as if set forth in their entireties. In the event of a conflict between a term as used herein and the term as defined in the incorporated reference, the definition of this disclosure controls.

SUMMARY

[0003] One embodiment provides a virus that produces from its genome a non-replicating retrovirus, wherein the virus is an oncolytic virus. In some embodiments, wherein the non-replicating retrovirus comprises a transgene that codes for a non-retroviral protein. One embodiment provides a virus that produces from its genome a non-replicating retrovirus, wherein the virus is an oncolytic virus, wherein the non-replicating retrovirus comprises a transgene that codes for a non-retroviral protein, and wherein the non-replicating retrovirus is capable of expressing the non-retroviral protein in cells that are infected by the oncolytic virus and in cells that are not infected by the oncolytic virus, in a tumor microenvironment.

[0004] One embodiments an oncolytic virus that produces from its genome a non-replicating retrovirus, wherein the non-replicating retrovirus comprises a transgene that codes for a non-lentiviral protein, and wherein the non-replicating retrovirus is capable of infecting and transducing both dividing and non-dividing cells in a target population of mammalian cells. One embodiment provides a virus comprising a transgene that codes for a non-retroviral protein, wherein the transgene is within an exogenous nucleic acid construct that further comprises a gene that codes for a protein of a non-replicating retrovirus, and wherein the virus is oncolytic. One embodiment provides a virus comprising a retroviral envelope construct, a retroviral packing construct, and a retroviral transfer construct, wherein at least one of the envelope construct, the packaging construct, or the transfer construct comprises a transgene that codes for a non-retroviral protein, and wherein the virus is an oncolytic virus.

[0005] In some embodiments, wherein the envelope construct, the packing construct, and the transfer construct are inserted at different locations within the genome of the oncolytic virus. In some embodiments, the envelope construct comprises a gene that codes for a retroviral envelope protein and the packaging construct comprises a gene that codes for a retroviral structural protein. In some embodiments, the gene that codes for the retroviral envelope protein is under the control of a first early viral promoter and the gene that codes for the retroviral structural protein is under the control of a second early viral promoter. In some embodiments, the gene that codes for the retroviral envelope protein is under the control of a first early viral promoter, a first early/late viral promoter or a first late viral promoter; and the gene that codes for a retroviral structural protein is under the control of a second early viral promoter, a second early/late viral promoter, or a second late viral promoter. In some embodiments, the envelope construct further comprises a termination sequence at its 5'-terminal region. In some embodiments, the packaging construct further comprises a termination sequence at its 3'-terminal region. In some embodiments, the transfer construct comprises a 3'-long terminal repeat region. In some embodiments, the transfer construct comprises a hybrid 5'-long terminal repeat region. In some embodiments, the hybrid 5'-long terminal repeat region comprises a third early viral promoter and a HIV-1 protein 5'-long terminal repeat region. In some embodiments, the hybrid 5'-long terminal repeat region comprises a HIV-1 protein 5'-long terminal repeat region and a viral promoter, wherein the viral promoter is a third early viral promoter, a third early/late viral promoter, or a third late viral promoter. In some embodiments, the transfer construct further comprises a gene that codes for a self-cleaving RNA ribozyme. In some embodiments, the gene that codes for the self-cleaving RNA ribozyme is positioned downstream to the 3'-long terminal repeat region of the transfer construct. In some embodiments, the self-cleaving ribozyme comprises a delta virus ribozyme or a hammerhead ribozyme. In some embodiments, the packaging construct comprises a 5'-long terminal repeat region and wherein the second early viral promoter is fused to the 5'-long terminal repeat region.

[0006] In some embodiments, the envelope construct comprises a termination sequence at its 5'-terminal region. In some embodiments, the packaging construct comprises a termination sequence at its 3'-terminal region. In some embodiments, the transfer construct comprises a termination sequence at its 3'-terminal region. In some embodiments, the termination sequence comprises a nucleotide sequence as set forth in SEQ ID No. 7. In some embodiments, the retroviral structural protein comprises a mature gag-pol protein. In some embodiments, the mature gag-pol protein comprises p55, p41, and p24. In some

embodiments, the retroviral envelope protein comprises a VSV-G protein. In some embodiments, the virus comprises an exogenous nucleic acid sequence that codes for a nucleic acid polymerase. In some embodiments, the nucleic acid polymerase is selected from the group consisting of: T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase, a RNA polymerase variant, and a DNA polymerase mutant. In some embodiments, the virus comprises an exogenous nucleic acid sequence that codes for a bacteriophage polymerase. In some embodiments, the bacteriophage is selected from the group consisting of a T3 bacteriophage, a T7 bacteriophage and an SP6 bacteriophage. In some embodiments, the T3 bacteriophage polymerase is expressed with a T3 bacteriophage promoter, a T7 bacteriophage polymerase is expressed with a T7 bacteriophage promoter, and an SP6 bacteriophage polymerase is expressed with an SP6 bacteriophage promoter. In some embodiments, the virus comprises an exogenous nucleic acid sequence that codes for a nucleic acid polymerase. In some embodiments, the nucleic acid polymerase is selected from the group consisting of: T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase, a RNA polymerase variant, and a DNA polymerase mutant. In some embodiments, the virus comprises an exogenous nucleic acid sequence that codes for a bacteriophage polymerase. In some embodiments, the bacteriophage is selected from the group consisting of a T3 bacteriophage, a T7 bacteriophage and an SP6 bacteriophage. In some embodiments, the T3 bacteriophage polymerase is expressed with a T3 bacteriophage promoter, a T7 bacteriophage polymerase is expressed with a T7 bacteriophage promoter, and an SP6 bacteriophage polymerase is expressed with an SP6 bacteriophage promoter.

[0007] One embodiment provides a virus comprising: (i) a retroviral envelope construct inserted at a first location within the genome of the virus; and (ii) a retroviral packaging construct inserted at a second location within the genome of the virus, wherein the first location and the second location are not contiguous, wherein the envelope construct comprises a nucleic acid that codes for a retroviral envelope protein, wherein the packaging construct comprises a nucleic acid that codes for a retroviral structural protein, and wherein the virus is an oncolytic virus. In some embodiments, the virus comprises (iii) a transfer construct inserted at a third location within the genome of the oncolytic virus. In some embodiments, the transfer construct comprises a transgene that codes for a non-retroviral protein. In some embodiments, the transfer construct is inserted near the 3' end of the genome of the oncolytic virus. In some embodiments, the gene that codes for the retroviral envelope protein is under the control of a first early viral promoter and the gene that codes for the retroviral structural protein is under the control of a second early viral promoter. In some

embodiments, the gene that codes for the retroviral envelope protein is under the control of a first early viral promoter, a first early/late viral promoter or a first late viral promoter; and the gene that codes for a retroviral structural protein is under the control of a second early viral promoter, a second early/late viral promoter, or a second late viral promoter. In some embodiments, the transfer construct comprises a hybrid 5'-long terminal repeat region. In some embodiments, the hybrid 5'-long terminal repeat region comprises a third early viral promoter and a HIV-1 protein 5'-long terminal repeat region. In some embodiments, the hybrid 5'-long terminal repeat region comprises a HIV-1 protein 5'-long terminal repeat region and a viral promoter, wherein the viral promoter is a third early viral promoter, a third early/late viral promoter, or a third late viral promoter. In some embodiments, the transfer construct further comprises a gene that codes for a self-cleaving RNA ribozyme. In some embodiments, the gene that codes for the self-cleaving RNA ribozyme is positioned downstream to the 3'-long terminal repeat region of the transfer construct. In some embodiments, the self-cleaving ribozyme comprises a delta virus ribozyme or a hammerhead ribozyme. In some embodiments, the packaging construct comprises a 5'-long terminal repeat region and wherein the second early viral promoter is fused to the 5'-long terminal repeat region. In some embodiments, the nucleic acid that codes for the retroviral envelope protein comprises a nucleotide sequence as set forth in SEQ ID No. 1, or a nucleotide sequence that is at least about 70% to at least about 99% identical to the sequence set forth as SEQ ID No. 1.

[0008] In some embodiments, the nucleic acid that codes for the retroviral structural protein comprises a nucleotide sequence as set forth in SEQ ID No. 2, or a nucleotide sequence that is at least about 70% to at least about 99% identical to the sequence set forth as SEQ ID No. 2. In some embodiments, the transfer construct comprises a 3'-long terminal repeat region. In some embodiments, the envelope construct comprises a termination sequence at its 5'-terminal region. In some embodiments, the packaging construct comprises a termination sequence at its 3'-terminal region. In some embodiments, the transfer construct comprises a termination sequence at its 3'-terminal region. In some embodiments, the termination sequence comprises nucleotide sequence as set forth in SEQ ID No. 5. In some embodiments, the first location and the second location are separated by at least about 10,000 bases. In some embodiments, the first location and the third location are separated by at least about 10,000 bases. In some embodiments, the second location and the third location are separated by at least about 10,000 bases. In some embodiments, the structural protein comprises a mature gag-pol protein. In some embodiments, the mature gag-pol protein comprises p55, p41, and

p24. In some embodiments, the envelope protein comprises a VSV-G protein. In some embodiments, the oncolytic virus is tumor selective in replication. In some embodiments, the non-replicating retrovirus is produced selectively within a tumor microenvironment. In some embodiments, the oncolytic virus in an oncolytic vaccinia virus. In some embodiments, the first, the second, and the third early viral promoters, wherein the first early viral promoter is a first vaccinia virus early promoter, the second early viral promoter is a second vaccinia virus early promoter, and the third early viral promoter is a third vaccinia virus early promoter. In some embodiments, the first vaccinia virus early promoter comprises a sequence as set forth in SEQ ID No. 9. In some embodiments, the second vaccinia virus early promoter comprises a sequence as set forth in SEQ ID No. 10. In some embodiments, the third vaccinia virus early promoter comprises a sequence set forth as SEQ ID No. 11. In some embodiments, the transfer construct comprises a human PGK promoter (SEQ ID No. 12). In some embodiments, the envelope construct is inserted between genes vacwr032 and vacwr033 of the oncolytic vaccinia virus. In some embodiments, the packaging construct is inserted between genes vacwr093 and vacwr095 of the oncolytic vaccinia virus. In some embodiments, the genome of the oncolytic vaccinia virus comprises a deletion of the vacwr094 gene, and wherein the packaging construct is inserted into the location of the vacwr094 gene. In some embodiments, the transfer construct is inserted between genes vacwr205 and vacwr206 of the oncolytic vaccinia virus. In some embodiments, the non-retroviral protein comprises a therapeutic protein or a diagnostic protein. In some embodiments, the non-retroviral protein comprises the therapeutic protein, and wherein the therapeutic protein comprises an immune checkpoint modulator, an antibody or portion thereof, a Fc fusion protein, an anticoagulant, a blood factor, a bone morphogenetic protein, an immunosuppressive agent, an immunostimulatory agent, an enzyme, a growth factor, a hormone, an interferon, an interleukin, a thrombolytic, an anti-angiogenic, a chemotherapeutic, an antibiotic, an antifungal, an antiviral, and any combination thereof.

[0009] In some embodiments, the retrovirus is an Alpharetrovirus, a Betaretrovirus, a Deltaretrovirus, an Epsilonretrovirus, a Gamma retrovirus, or a Lentivirus. In some embodiments, the retrovirus is the lentivirus. In some embodiments, the lentivirus is an HIV. In some embodiments, the retrovirus is the Gamma retrovirus. In some embodiments, the Gamma retrovirus is a Moloney murine leukemia virus. In some embodiments, the virus comprises an exogenous nucleic acid sequence that codes for a nucleic acid polymerase. In some embodiments, the nucleic acid polymerase is selected from the group consisting of: T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase, a RNA polymerase

variant, and a DNA polymerase mutant. In some embodiments, the virus comprises an exogenous nucleic acid sequence that codes for a bacteriophage polymerase. In some embodiments, the bacteriophage is selected from the group consisting of a T3 bacteriophage, a T7 bacteriophage and an SP6 bacteriophage. In some embodiments, the T3 bacteriophage polymerase is expressed with a T3 bacteriophage promoter, a T7 bacteriophage polymerase is expressed with a T7 bacteriophage promoter, and an SP6 bacteriophage polymerase is expressed with an SP6 bacteriophage promoter.

[0010] One embodiment a method of treating cancer, the method comprising: administering to a subject an oncolytic virus according to this disclosure, wherein the non-replicating retrovirus generated from the oncolytic virus is selectively targeted to a tumor tissue. In some embodiments, the tumor tissue comprises a malignant neoplastic tissue. One embodiment provides an engineered producer virus that generates a non-replicating retrovirus from its genome, wherein the engineered producer virus comprises at least one of the following modifications:

i. mutation or deletion of at least one viral gene;
ii. insertion of at least one exogenous nucleic acid;
iii. altered tropism; or
iv. any combinations thereof,

and wherein the non-replicating retrovirus comprises a transgene that codes for a non-retroviral protein. In some embodiments, the non-replicating lentivirus comprises a transgene that codes for a non-retroviral protein. In some embodiments, the producer virus is a vaccinia virus and the at least one viral gene is selected from the group consisting of: B5R, A52R (VACWR178), F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, C12L, TK, and any combinations thereof. In some embodiments, the at least one exogenous nucleic acid codes for a protein selected from the group consisting of: CXCR4, CCR2, PH-20, HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, a metabolic modulating protein, a cytokine, a fusion protein comprising any combinations of the above, and a functional domain or fragment or variant thereof, or any combinations thereof. In some embodiments, the at least one exogenous nucleic acid codes for the PIAS3.

[0011] In some embodiments, the producer virus comprises a retroviral envelope construct, a retroviral packaging construct and a retroviral transfer construct, wherein the envelope construct, the packing construct, and the transfer construct are inserted at different locations

within the genome of the producer virus. In some embodiments, the envelope construct comprises a gene that codes for a retroviral envelope protein and the packaging construct comprises a gene that codes for a retroviral structural protein. In some embodiments, the gene that codes for the retroviral envelope protein is under the control of a first early viral promoter and the gene that codes for the retroviral structural protein is under the control of a second early viral promoter. In some embodiments, the envelope construct further comprises a termination sequence at its 5'-terminal region.

[0012] In some embodiments, the packaging construct further comprises a termination sequence at its 3'-terminal region. In some embodiments, the transfer construct comprises a termination sequence. In some embodiments, the transfer construct comprises a hybrid 5'-long terminal repeat region. In some embodiments, the hybrid 5'-long terminal repeat region comprises a third early viral promoter and a HIV-1 protein 5'-long terminal repeat region. In some embodiments, the packaging construct comprises a 5'-long terminal repeat region and wherein the second early viral promoter is fused to the 5'-long terminal repeat region. In some embodiments, the envelope construct comprises a termination sequence at its 5'-terminal region. In some embodiments, the packaging construct comprises a termination sequence at its 3'-terminal region. In some embodiments, the transfer construct comprises a termination sequence. In some embodiments, the termination sequence comprises a nucleotide sequence as set forth in SEQ ID No. 7. In some embodiments, the retroviral structural protein comprises a mature gag-pol protein. In some embodiments, the mature gag-pol protein comprises p55, p41, and p24. In some embodiments, the retroviral envelope protein comprises a VSV-G protein. In some embodiments, the producer virus in an oncolytic vaccinia virus.

[0013] In some embodiments, the envelope construct comprises a first early vaccinia promoter, the packaging construct comprises a second early vaccinia promoter, and the transfer construct comprises a third early vaccinia promoter. In some embodiments, the first vaccinia virus early promoter comprises a sequence as set forth in SEQ ID No. 9. In some embodiments, the second vaccinia virus early promoter comprises a sequence as set forth in SEQ ID No. 10. In some embodiments, the third vaccinia virus early promoter comprises a sequence set forth as SEQ ID No. 11. In some embodiments, the transfer construct comprises a human PGK promoter (SEQ ID No. 12). In some embodiments, the envelope construct is inserted between genes vacwr032 and vacwr033 of the oncolytic vaccinia virus. In some embodiments, the packaging construct is inserted between genes vacwr093 and vacwr095 of the oncolytic vaccinia virus. In some embodiments, the genome of the oncolytic vaccinia

virus comprises a deletion of the vacwr094 gene, and wherein the packaging construct is inserted into the location of the vacwr094 gene. In some embodiments, the transfer construct is inserted between genes vacwr205 and vacwr206 of the oncolytic vaccinia virus. In some embodiments, the non-retroviral protein comprises a therapeutic protein or a diagnostic protein. In some embodiments, the virus comprises the transgene that codes for the non-retroviral protein, wherein the non-retroviral protein comprises the therapeutic protein, and wherein the therapeutic protein comprises an immune checkpoint modulator, an antibody or portion thereof, a Fc fusion protein, an anticoagulant, a blood factor, a bone morphogenetic protein, an immunosuppressive agent, an immunostimulatory agent, an enzyme, a growth factor, a hormone, an interferon, an interleukin, a thrombolytic, an anti-angiogenic, a chemotherapeutic, an antibiotic, an antifungal, an antiviral, and any combination thereof. In some embodiments, the retrovirus is an Alpharetrovirus, a Betaretrovirus, a Deltaretrovirus, an Epsilonretrovirus, a Gamma retrovirus, or a Lentivirus. In some embodiments, the retrovirus is a lentivirus. In some embodiments, the lentivirus is an HIV. In some embodiments, the retrovirus is the Gamma retrovirus. In some embodiments, the Gamma retrovirus is a Moloney murine leukemia virus (MMLV).

[0014] One embodiment provides a method of treating cancer, the method comprising: administering to a subject an engineered producer virus according to this disclosure, wherein the non-replicating retrovirus generated from the engineered producer virus is selectively targeted to a tumor tissue. In some embodiments, the tumor tissue comprises a malignant neoplastic tissue.

[0015] One embodiment provides a process for generating an oncolytic virus that produces a non-replicating retrovirus, the process comprising: growing a population of the oncolytic virus in mammalian cells, followed by adding and selecting for, sequentially, a retroviral envelope construct, a retroviral packaging construct, and a retroviral transfer construct, wherein at least one of the envelope construct, the packaging construct, or the transfer construct comprises a transgene that codes for a non-retroviral protein. In some embodiments, the retrovirus is an Alpharetrovirus, a Betaretrovirus, a Deltaretrovirus, an Epsilonretrovirus, a Gamma retrovirus, or a Lentivirus. In some embodiments, the retrovirus is the Gamma retrovirus. In some embodiments, the Gamma retrovirus is a Moloney murine leukemia virus. In some embodiments, the retrovirus is the Lentivirus. In some embodiments, the Lentivirus is HIV.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of this disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of this disclosure are utilized, and the accompanying drawings of which.

[0017] **FIG. 1** shows locations of insertions or insertion/deletions of each lentiviral construct within the vaccinia virus genome.

[0018] **FIG. 2** shows a map of the lentiviral *env* construct.

[0019] **FIG. 3** shows a map of the lentiviral packaging construct.

[0020] **FIG. 4** shows a map of the lentiviral transfer construct.

[0021] **FIG. 5** shows detection of *GAG* expression by Western blot.

[0022] **FIG. 6** shows detection of VSV-G expression by Western blot.

[0023] **FIG. 7** shows a fluorescent microscopy image of HEK293 cells infected with Lenti-Vac virus generating infectious lentivirus, as shown by the presence of TagRFP-T and sfGFP on the cells; indicating vaccinia virus infection and lentivirus transduction respectively.

[0024] **FIG. 8** shows an exemplary construct containing a T7 RNA polymerase sequence.

DETAILED DESCRIPTION

[0025] While preferred embodiments of this disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from this disclosure. It should be understood that various alternatives to the embodiments of this disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Certain Definitions

[0026] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms "a", "an" and "the" can include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "contains," "containing," "including", "includes," "having," "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising."

[0027] The term "about" or "approximately" can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *e.g.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the given value. Where particular values are described in the application and claims, unless otherwise stated the term "about" should be assumed to mean an acceptable error range for the particular value, such as ±10% of the value modified by the term "about".

[0028] The terms "individual," "patient," or "subject" can be used interchangeably. None of the terms require or are limited to situation characterized by the supervision (*e.g.* constant or intermittent) of a health care worker (*e.g.* a doctor, a registered nurse, a nurse practitioner, a physician's assistant, an orderly, or a hospice worker). In some embodiments, patients, subjects, or individuals can be under the supervision of a health care worker.

[0029] The terms "heterologous nucleic acid sequence," or "exogenous nucleic acid sequence," or "transgenes," as used herein, in relation to a specific virus can refer to a nucleic acid sequence that originates from a source other than the specified virus.

[0030] The term "mutation," as used herein, can refer to a deletion, an insertion of a heterologous nucleic acid, an inversion or a substitution, including an open reading frame ablating mutations as commonly understood in the art.

[0031] The term "cancer" and its grammatical equivalents as used herein can refer to a hyperproliferation of cells whose unique trait—loss of normal controls—results in unregulated growth, lack of differentiation, local tissue invasion, and metastasis. With respect to the inventive methods, the cancer can be any cancer, including any of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bladder cancer, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, rectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, fibrosarcoma, gastrointestinal carcinoid tumor, Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, leukemia, liquid tumors, liver cancer, lung cancer, lymphoma, malignant mesothelioma, mastocytoma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer, skin cancer, small intestine cancer, soft tissue cancer, solid tumors, stomach cancer, testicular cancer, thyroid cancer, ureter cancer, and/or urinary bladder cancer. As

used herein, the term "tumor" refers to an abnormal growth of cells or tissues, *e.g.*, of malignant type or benign type.

[0032] The term "gene," as used herein, can refer to a segment of nucleic acid that encodes an individual protein or RNA (also referred to as a "coding sequence" or "coding region"), optionally together with associated regulatory regions such as promoters, operators, terminators and the like, which may be located upstream or downstream of the coding sequence.

[0033] The terms "mutant virus" and "modified virus," as used interchangeably herein, can refer to a virus comprising one or more mutations in its genome, including but not limited to deletions, insertions of heterologous nucleic acids, inversions, substitutions or combinations thereof.

[0034] The term "naturally-occurring," as used herein with reference to a virus, can indicate that the virus can be found in nature, *i.e.*, it can be isolated from a source in nature and has not been intentionally modified.

[0035] The terms "inhibiting," "reducing" or "prevention," or any variation of these terms, referred to herein, can include any measurable decrease or complete inhibition to achieve a desired result.

[0036] A "promoter," as used herein, can be a control sequence that is a region of a nucleic acid sequence at which initiation and rate of transcription are controlled. In certain embodiments, a promoter may contain genetic elements at which regulatory proteins and molecules may bind such as RNA polymerase and other transcription factors. The terms "operatively positioned," "operatively linked," "under control" and "under transcriptional control" can mean that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence to control transcriptional initiation and/or expression of that sequence. In certain embodiments, a promoter may or may not be used in conjunction with an "enhancer," which refers to a cis-acting regulatory sequence involved in the transcriptional activation of a nucleic acid sequence.

[0037] The term "homology," as used herein, may be to calculations of "homology" or "percent homology" between two or more nucleotide or amino acid sequences that can be determined by aligning the sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first sequence). The nucleotides at corresponding positions may then be compared, and the percent identity between the two sequences may be a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100). For example, a position in the first sequence

may be occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent homology between the two sequences may be a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. In some embodiments, the length of a sequence aligned for comparison purposes may be at least about: 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 95%, of the length of the reference sequence. A BLAST® search may determine homology between two sequences. The homology can be between the entire lengths of two sequences or between fractions of the entire lengths of two sequences. The two sequences can be genes, nucleotides sequences, protein sequences, peptide sequences, amino acid sequences, or fragments thereof. The actual comparison of the two sequences can be accomplished by well-known methods, for example, using a mathematical algorithm. A non-limiting example of such a mathematical algorithm may be described in Karlin, S. and Altschul, S., Proc. Natl. Acad. Sci. USA, 90-5873-5877 (1993). Such an algorithm may be incorporated into the NBLAST and XBLAST programs (version 2.0), as described in Altschul, S. et al., Nucleic Acids Res., 25:3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, any relevant parameters of the respective programs (*e.g.*, NBLAST) can be used. For example, parameters for sequence comparison can be set at score= 100, word length= 12, or can be varied (*e.g.*, W=5 or W=20). Other examples include the algorithm of Myers and Miller, CABIOS (1989), ADVANCE, ADAM, BLAT, and FASTA. In another embodiment, the percent identity between two amino acid sequences can be accomplished using, for example, the GAP program in the GCG software package (Accelrys, Cambridge, UK).

[0038] The term "subject" can refer to an animal, including, but not limited to, a primate (*e.g.*, human), cow, sheep, goat, horse, dog, cat, rabbit, rat, or mouse. The terms "subject" and "patient" are used interchangeably herein in reference, for example, to a mammalian subject, such as a human subject.

[0039] The terms "treat," "treating," and "treatment" can be meant to include alleviating or abrogating a disorder, disease, or condition; or one or more of the symptoms associated with the disorder, disease, or condition; or alleviating or eradicating the cause(s) of the disorder, disease, or condition itself. Desirable effects of treatment can include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishing any direct or indirect pathological consequences of the disease, preventing metastasis,

decreasing the rate of disease progression, amelioration or palliation of the disease state and remission or improved prognosis.

[0040] The term "therapeutically effective amount" can refer to the amount of a compound that, when administered, can be sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of the disorder, disease, or condition being treated. The term "therapeutically effective amount" can also refer to the amount of a compound that is sufficient to elicit the biological or medical response of a cell, tissue, system, animal, or human that is being sought by a researcher, veterinarian, medical doctor, or clinician.

[0041] The term "pharmaceutically acceptable carrier," "pharmaceutically acceptable excipient," "physiologically acceptable carrier," or "physiologically acceptable excipient" can refer to a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, solvent, or encapsulating material. A component can be "pharmaceutically acceptable" in the sense of being compatible with the other ingredients of a pharmaceutical formulation. It can also be suitable for use in contact with the tissue or organ of humans and animals without excessive toxicity, irritation, allergic response, immunogenicity, or other problems or complications, commensurate with a reasonable benefit/risk ratio. S*ee, Remington: The Science and Practice of Pharmacy*, 21st Edition; Lippincott Williams & Wilkins: Philadelphia, PA, 2005; *Handbook of Pharmaceutical Excipients*, 5th Edition; Rowe et al., Eds., The Pharmaceutical Press and the American Pharmaceutical Association: 2005; and *Handbook of Pharmaceutical Additives*, 3rd Edition; Ash and Ash Eds., Gower Publishing Company: 2007; *Pharmaceutical Preformulation and Formulation*, Gibson Ed., CRC Press LLC: Boca Raton, FL, 2004).

[0042] The term "pharmaceutical composition" can refer to a mixture of a compound disclosed herein with other chemical components, such as diluents or carriers. The pharmaceutical composition can facilitate administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but not limited to, oral, injection, aerosol, parenteral, and topical administration. Pharmaceutical compositions can also be obtained by reacting compounds with inorganic or organic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

[0043] An "anti-cancer agent," as used herein, can refer to an agent or therapy that is capable of negatively affecting cancer in a subject, for example, by killing cancer cells, inducing apoptosis in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases, reducing tumor size, inhibiting tumor growth,

reducing the blood supply to a tumor or cancer cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer. Non-limiting examples of anti-cancer agents can include biological agents (biotherapy), chemotherapy agents, and radiotherapy agents.

[0044] The term "oncolytic," as used herein, can refer to killing of cancer or tumor cells by an agent, such as an oncolytic pox virus, such as an oncolytic vaccinia virus, *e.g.*, through the direct lysis of said cells, by stimulating immune response towards said cells, apoptosis, expression of toxic proteins, autophagy and shut-down of protein synthesis, induction of anti-tumoral immunity, or any combinations thereof. The direct lysis of the cancer or tumor cells infected by the agent, such as an oncolytic vaccinia virus, can be a result of replication of the virus within said cells. In certain examples, the term "oncolytic," can refer to killing of cancer or tumor cells without lysis of said cells.

[0045] The term "oncolytic virus" as used herein can refer to a virus that preferentially infects and kills tumor cells. Under certain non-limiting circumstances, it is understood that oncolytic viruses can promote anti-tumor responses through dual mechanisms dependent on not only the selective killing of tumor cells, but also the stimulation of host anti-tumor immune responses. In some embodiments, the oncolytic viruses can include, but are not limited to, (i) viruses that naturally replicate preferentially in cancer cells and are non-pathogenic in humans often due to elevated sensitivity to innate antiviral signaling or dependence on oncogenic signaling pathways; and (ii) viruses that are genetically-manipulated for use. In some embodiments, the oncolytic virus can be a measles virus, a poliovirus, a poxvirus, a vaccinia virus, an adenovirus, an adeno associated virus, a herpes simplex virus, a vesicular stomatitis virus, a reovirus, a Newcastle disease virus, a senecavirus, a lentivirus, a mengovirus, or a myxomavir. In certain embodiments, the oncolytic virus can be a pox virus. In certain embodiments, the oncolytic virus can be a vaccinia virus.

[0046] The term "modified oncolytic virus" as used herein can refer to an oncolytic virus that comprises a modification to its constituent, such as, but not limited to, a modification in the native genome ("backbone") of the virus like a mutation or a deletion of a viral gene, introduction of an exogenous nucleic acid, a chemical modification of a viral nucleic acid or a viral protein, and introduction of a exogenous protein or modified viral protein to the viral capsid. In general, oncolytic viruses may be modified (also known as "engineered") in order to gain improved therapeutic effects against tumor cells. In certain embodiments, the

modified oncolytic virus can be a modified pox virus. In certain embodiments, the modified oncolytic virus can be a modified pox virus.

[0047] The terms "systemic delivery," and "systemic administration," used interchangeably herein, in some cases can refer to a route of administration of medication, oncolytic virus or other substances into the circulatory system. The systemic administration may comprise oral administration, parenteral administration, intranasal administration, sublingual administration, rectal administration, transdermal administration, or any combinations thereof.

[0048] As used herein the terms "replication deficient virus" or "non-replicating virus," or "replication incompetent virus," have their ordinary meaning, such as, a virus that is propagation incompetent as a result of modifications to its genome. Thus, once such recombinant virus infects a cell, the only course it can follow is to express any viral and heterologous protein contained in its genome. In a specific embodiment, the replication defective vectors provided herein may contain genes encoding nonstructural proteins and are self-sufficient for RNA transcription and gene expression. However, these vectors lack genes encoding structural proteins, so that a helper genome is needed to allow them to be packaged into infectious particles. In addition to providing therapeutically safe vectors, the removal of the structural proteins increases the capacity of these vectors to incorporate more than 6 kb of heterologous sequences. In another embodiment, propagation incompetence of the adenovirus vectors of the invention is achieved indirectly, e.g., by removing the packaging signal which allows the structural proteins to be packaged in virions being released from the packaging cell line.

[0049] In an aspect, provided herein is a producer virus that can deliver a cargo that comprises a replication defective virus, for instance, a replication defective virus of a different family than the producer virus. In an aspect, the producer virus can be an oncolytic virus, for example a vaccinia virus that produces from its genome a non-replicating virus, such as a non-replicating lentivirus. In some cases, a cargo can be at least a portion of a non-replicating lentivirus (LV). In some cases, a non-replicating lentivirus can comprise a transgene such as a non-lentiviral protein. A non-lentiviral protein can be a therapeutic protein or functional fragment thereof and/or a diagnostic protein. In some cases, the non-replicating lentivirus is capable of expressing the non-lentiviral protein in cells that are infected by the oncolytic virus leading to lysis of the infected cell, such as a cancer cell. In some cases, the non-replicating virus can be produced by the producer virus *in situ*, such as within a tumor cell or in a tumor micro-environment. For example, the non-replicating virus

can be produced in a tumor cell that is infected by the oncolytic producer virus, and the non-replicating virus can express a therapeutic or a diagnostic protein *in situ*.

[0050] In some aspects, provided herein can be a vector system comprising a producer oncolytic virus. In some aspects, a vector system can comprise a disrupted viral genome. In some aspects, a single vector can comprise at least two viral genomes. In some aspects, a single vector can comprise at least 3 viral genomes. In an aspect, a producer oncolytic vaccinia virus genome can comprise a sequence from a foreign virus, such as an HIV virus. In some aspects, a 3-plasmid system can be provided herein in which the *env*, packaging, and transfer functions of a virus can be on 3 different plasmids. In some aspects, *env*, packaging and transfer may be on a single vector comprising at least 1 viral genome.

[0051] In some examples, a producer virus can be from a family of any one of: Myoviridae, Podoviridae, Siphoviridae, Alloherpesviridae, Herpesviridae, Malacoherpesviridae, Lipothrixviridae, Rudiviridae, Adenoviridae, Ampullaviridae , Ascoviridae, Asfaviridae, Baculoviridae , Bicaudaviridae, Clavaviridae, Corticoviridae, Fuselloviridae, Globuloviridae, Guttaviridae, Hytrosaviridae, Iridoviridae, Marseilleviridae, Mimiviridae, Nimaviridae, Pandoraviridae, Papillomaviridae, Phycodnaviridae, Plasmaviridae, Polydnaviruses, Polyomaviridae, Poxviridae, Sphaerolipoviridae, or Tectiviridae. In an aspect, provided herein a producer oncolytic virus can be a poxviridae virus, such as a vaccinia virus. In an aspect, a vaccinia virus can encode a virus from a different family. For example, a vaccinia virus genome can comprise a second genome for a virus selected from a virus of any of the following families: Myoviridae, Podoviridae, Retroviridae, Siphoviridae, Alloherpesviridae, Herpesviridae, Malacoherpesviridae, Lipothrixviridae, Rudiviridae, Adenoviridae, Ampullaviridae , Ascoviridae, Asfaviridae, Baculoviridae , Bicaudaviridae, Clavaviridae, Corticoviridae, Fuselloviridae, Globuloviridae, Guttaviridae, Hytrosaviridae, Iridoviridae, Marseilleviridae, Mimiviridae, Nimaviridae, Pandoraviridae, Papillomaviridae, Phycodnaviridae, Plasmaviridae, Polydnaviruses, Polyomaviridae, Sphaerolipoviridae, or Tectiviridae.

[0052] In some embodiments, a virus produced in situ can be a retrovirus (including lentiviruses), herpes viruses, alphavirus, adeno-associated viruses, vaccinia virus, papillomavirus, or Epstein Barr virus (EBV). In accordance with the present disclosure there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. In certain embodiments, a virus produced by the producer virus of this disclosure is replication defective, that is, it may be unable to replicate autonomously in a target cell. Preferably, a replication defective virus is a minimal virus,

such that it retains only the sequences of its genome which are necessary for target cell recognition and encapsidating the viral genome. Replication defective virus may not be infective after introduction into a cell. Use of replication defective viral vectors may allow for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Thus, a specific tissue can be specifically targeted, for example a cancer cell.

[0053] In an aspect, a virus produced from a producer virus provided herein can be from the family Retroviridae. In an aspect, a Retroviridae virus can be any one of: an Alpharetrovirus, a Betaretrovirus, a Deltaretrovirus, an Epsilonretrovirus, a Gamma retrovirus (e.g., a Moloney murine leukemia virus), or a Lentivirus. In some embodiments, further specific examples of a retrovirus can be any one of: HIV- 1, HIV-2, HTLV-1, HTLV-2, HTLV-3, HTLV-4, equine infectious anemia virus (EIAV) lentivirus, or a combination thereof.

[0054] The retroviruses produced by the producer viruses disclosed herein are, in some embodiments, integrating viruses which can infect dividing cells as well as non-dividing cells. The retrovirus genome can have two LTRs, an encapsidation sequence and three coding regions (*gag*, *pol* and *env*). Replication defective non-infectious retroviral vectors can be manipulated to destroy the viral packaging signal but can retain the structural genes required to package the co-introduced virus engineered to contain the heterologous gene and the packaging signals. Thus, in recombinant replication defective retroviral vectors, the *gag*, *pol* and *env* genes may generally be deleted, in whole or in part, and replaced with a heterologous nucleic acid sequence of interest. These vectors can be constructed from different types of retroviruses, such as HIV (human immuno-deficiency virus), MoMuLV (murine Moloney leukaemia virus), MSV (murine Moloney sarcoma virus), HaSV (Harvey sarcoma virus), SNV (spleen necrosis virus), RSV (Rous sarcoma virus), and Friend virus.

[0055] In a specific embodiment of the disclosure, replication defective lentiviruses produced from an oncolytic producer virus can be used as agents for the direct delivery and sustained expression of a transgene in several tissue types, including brain, retina, muscle, liver, and blood. This subtype of retroviral vectors can efficiently transduce dividing and nondividing cells in these tissues and maintain long-term expression of the gene of interest (for a review, see, Naldini, Curr. Opin. Biotechnol. 1998, 9:457-63; Zufferey, et al., J. Virol. 1998, 72:9873-80). Lentiviral packaging cell lines are available and known generally in the art (see, e.g., Kafri, et al., J. Virol., 1999, 73: 576-584).

[0056] In an aspect, a producer virus or a virus produced in situ can also be an adenovirus. Adenoviruses are eukaryotic DNA viruses that can be modified to efficiently deliver a nucleic acid of the disclosure to a variety of cell types. Various serotypes of adenovirus exist,

such as type 2 or type 5 human adenoviruses (Ad 2 or Ad 5) or adenoviruses of animal origin (see PCT Publication No. WO94/26914). Those adenoviruses of animal origin can include adenoviruses of canine, bovine, murine (e.g., Mav1 [Beard et al., Virology, 1990, 75:81]), ovine, porcine, avian, and simian (e.g., SAV) origin. Preferably, the adenovirus of animal origin is a canine adenovirus, more preferably a CAV2 adenovirus (e.g., Manhattan or A26/61 strain [ATCC Accession No. VR-800]). Various replication defective adenovirus and minimum adenovirus vectors have been described (PCT Publications No. WO94/26914, WO95/02697, WO94/28938, WO94/28152, WO94/12649, WO95/02697, WO96/22378). The replication defective recombinant adenoviruses according to the disclosure can be prepared by any technique known to the person skilled in the art (Levrero et al., Gene, 1991, 101:195; EP Publication No. 185 573; Graham, EMBO J., 1984, 3:2917; Graham et al., J. Gen. Virol., 1977, 36:59). Recombinant adenoviruses are recovered and purified using standard molecular biological techniques, which are well known to one of ordinary skill in the art. Adeno-associated virus-based vectors. The adeno-associated viruses (AAV) are DNA viruses of relatively small size which can integrate, in a stable and site-specific manner, into the genome of the cells which they infect. They are able to infect a wide spectrum of cells without inducing any effects on cellular growth, morphology or differentiation, and they do not appear to be involved in human pathologies. The AAV genome has been cloned, sequenced and characterized. The use of vectors derived from the AAVs for transferring genes *in vitro* and *in vivo* has been described (see PCT Publications No. WO 91/18088 and WO 93/09239; U.S. Pat. Nos. 4,797,368 and 5,139,941; EP Publication No. 488 528). The replication defective recombinant AAVs according to the disclosure can be prepared by cotransfecting a plasmid containing the nucleic acid sequence of interest flanked by two AAV inverted terminal repeat (ITR) regions, and a plasmid carrying the AAV encapsidation genes (rep and cap genes), into a cell line which is infected with producer virus (e.g., an oncolytic vaccinia virus). The AAV recombinants which are produced can then be purified by standard techniques.

Oncolytic Virus

[0057] Provided herein can be an oncolytic producer virus or portion thereof. An oncolytic virus can be a virus that can infect and lyse a cancer cell. In an aspect, an oncolytic virus may have at least one of the following functions in cancer therapy: (1) directly destroying the tumor cells by viral lysis, (2) serving as a vector for expressing heterologous proteins in the tumor site, and (3) the presentation of autologous tumor antigens to prime/activate the immune system. Oncolytic virus selectivity for cancer cells can occur either during infection

or during replication. Tumor-selective viruses can be engineered by altering viral surface proteins that recognize specific cellular receptors, allowing the virus to specifically enter cancer cells. Replication selectivity can be accomplished by modifying the viral genes that are required for efficient replication, so that the virus can only replicate in cells that have disruptions in normal homeostatic pathways, such as tumor-suppressor defects or activation of oncogenic pathways.

[0058] In an aspect, an oncolytic virus can be any one of: a vesicular stomatitis virus (VSV), a Newcastle disease virus (NDV), a retrovirus, a reovirus, a measles virus, a Sinbis virus, an influenza virus, a herpes simplex virus, vaccinia virus, and an adenovirus. In some cases, an oncolytic virus can comprise a poxvirus. In some cases the poxvirus can comprises a measles virus, a poliovirus, a poxvirus, a vaccinia virus, an adenovirus, an adeno associated virus, a herpes simplex virus, a vesicular stomatitis virus, a reovirus, a Newcastle disease virus, a senecavirus, a lentivirus, a mengovirus, a myxomavir, a betaentomopoxvirus, a yatapoxvirus, a cervidpoxvirus, a gammaentomopoxvirus, a leporipoxvirus, a suipoxvirus, a molluscipoxvirus, a crocodylidpoxvirus, a alphaentomopoxvirus, a capripoxvirus, a avipoxvirus, or a parapoxvirus. In some cases, the poxvirus can comprise a vaccinia virus.

[0059] In one embodiment, the oncolytic producer virus can be a vaccinia virus. Vaccinia virus can be a large, complex enveloped virus having a linear double-stranded DNA genome of about 190 kilobases and encodes approximately 250 genes. Vaccinia is well-known for its role as a vaccine that eradicated smallpox. Vaccinia virus is unique among DNA viruses as it replicates only in the cytoplasm of the host cell. Therefore, the large genome is required to code for various enzymes and proteins needed for viral DNA replication. During replication, vaccinia produces several infectious forms which differ in their outer membranes: the intracellular mature virion (IMV), the intracellular enveloped virion (IEV), the cell-associated enveloped virion (CEV) and the extracellular enveloped virion (EEV). IMV is the most abundant infectious form and is thought to be responsible for spread between hosts. A number of genes in vaccinia can be modified in order to improve its properties as an oncolytic virus.

[0060] In some cases, the oncolytic producer virus can be Vaccinia virus western reserve (WR), the genome sequence of which is provided in **Table 1**. In an aspect, a vaccinia virus genome, such as the genome sequence provided in **Table 1**, can be disrupted with at least 1, 2, 3, 4, 5, 6, 7, or up to 8 exogenous sequences, e.g., exogenous sequences that code for a replication defective virus produced from the vaccinia virus. In an aspect, a producer

oncolytic virus provided herein can comprise percent identity from about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or up to about 100% to SEQ ID NO: 1.

[0061] **Table 1: Complete genome of Vaccinia Virus WR**

SEQ ID NO:	Sequence from GenBank: AY243312.1
1	ATTTAAAATATAATATTAATGTACTAAAACTTATATATTATTAATTTATCTAACTAAAGTTAGTAAATTA TATATATAATTTTATAATTAATTTAATTTTACTAATTTTATTTAGTGTCTAGAAAAAAATGTGTGACCCA TGACTGTAGGAAACTCTAGAGTGTAAGAAAGATCGATCGCTTTATAGAGACCATCAGAAAGAGGTTTAAT ATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTTACGACTCCATCAGAAAGAGGTTTAAT ATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTTACGACTCCATCAGAAAGAGGTTTAAT ATTTTTGTGAGACCATCGAAGGAGAAAGAGATAAAACTTTTTTACGACTCCATCAGAAAGAGGTTTAATA TTTTTGTGAGACCATCGAAGGAGAAAGAGATAAAACTTTTTTACGACTCCATCAGAAAGAGGTTTAATAT TTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTTACGACTCCATCAGAAAGAGGTTTAATAT TTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTTACGACTCCATCAGAAAGAGGTTTAATAT TTTTGTGAGACCATCGAAGGAGAAAGAGATAAAACTTTTTTACGACTCCATCAGAAAGAGGTTTAATATT TTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTTACGACTCCATCAGAAAGAGGTTTAATATT TTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTTACGACTCCATCAGAAAGAGGTTTAATATT TTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTTACGACTCCATCAGAAAGAGGTTTAATATT TTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTTACGACTCCATCAGAAAGAGGTTTAATATT TTTGTGAGACCATCGAAGAGAGAAAGAGAAAGAGATAGTTGATCTAGATATTTTTCTTAGTACAAAAGTC AATGTTTTAAAATATATGGACAAGAATTTGTCTGTATAAAAACTTGTGTGAAATTTTGTACCAAAGAAAA AATGTGAGCAGTATCCCCTACATGGATTTTACTAGATCATTTATATACCAAAAAATATTATACGATCTAC GTTTTATTATATGATTTTAACGTGTAAATTATAAACATTATTTTATGATATACAATTGTCTGGTAACCTA GATGGGCATAGGGGATGTTGATAAGCTCGACGAGTATATGTTGTTGGACGTTATTGTTTAAGAAATAGTT GATGCATCAGAAAGAGAATAAAAAATATTTTAGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGATAAAACTTTTTT ACGACTCCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGAATAAAAATATTT TATGACTCCATTGAAGAGAGAAAGAGAAAATGAGAATGAGAATAAAAATATTTTAGTGACACCATCAGAA AGAGGTTTAATATTTTTGTGAGACCATCGAAGAGAGAAAGAGAATAAAAATATTTTATGACTCCATTGAA GAGAGAAAGAGAAAATGAGAATGAGAATAAAAATATTTTAGTGACACCATCAGAAAGAGGTTTAATATTT TTTATGAGACCATCAAAGAGAGAAAGAGAATAAAAATATTTTTGTAAAACTTTTTTTATGAGACCATCAA AGAGAGAAAGAGAATAAAAATATTTTTGTAAAACTTTTTTTATGAGACCATCAAAGAGAGAAAGAGAATA AAAATATTTTTGTAAAACTTTTTTTATGAGACCATCAAAGAGAGAAAGAGAATAAAAATATTTTTGTAAA ACTTTTTTTATGAGACCATCAAAGAGAGAAAGAGAATAAAAATATTTTTGTAAAACTTTTTTTATGAGAC CATCAAAGAGAGAAAGAGAATAAAAATATTTTTGTAAAACTTTTTTTATGAGACCATCAAAGAGAGAAAG AGAATAAAAATATTTTTGTAAAACTTTTTTTATGAGACCATCAAAGAGAGAAAGAGAATAAAAATATTTT TGTAAAACTTTTTTTATGAGACCATCAGAAAGAGGTTTAATATTTTTGTGATACCCTGAAAGGAAATAGG AATAGGAATAGGAATAGTGTCATAATCGTATCACACTATTGAGACAGAAAAAGAAGAAGTCGCGAGAGGT AACTTTTTGTGAATGTAGTTAAGAACATTTTTGTTTTGCAAACCGGAATATAGTGTCCGGTACACTTTTT TAATTCGTGGTGTGCCTGAATCGTTCGATTAACCCTACTCATCCAATTTCAGATGAATAGAGTTATCGAT

	TCAGACACACGCTTTGAGTTTTGTTGAATCGATGAGTGAAGTATCATCGGTTGCACCTTCAGATGCCGAT
	CCGTCGACATACTTAAATCCATCCTTGACCTCAAGTTCAGATGATTCCTTGCACATGTCTCCGATACGAA
	CGCTAAACTCTAGATTCTTGACACATTTTGTATCGACGATCGTTGAACCGATGATATCTTCGTAACTCAC
	TTTCTTATGAGAGATGTTAGACCCGAGTACTGGATGGGTCTTGATGTCGCTGTCTTTCTCTTCTTCGCTA
	CATCTGATGTCGATAGACACCTCACAGTCTTTGATCATAGCCAGAGCTTCTTCATGAGTGATCGCGGGAG
	AGTCCTTACCTTGTCCTGGGGACACGCTGGACAATCTAGCATTCACTGTGTTTCCATCAGCGGATTCTGA
	GATGGATTTAATCTGAGGACATTTGGTGAATCCAAAGTTCATTCTCAGACCTCCACCGATGATGGAGTAA
	TAAGTGGTAGGAGGATCTACATCCTCGACTGATGTGGAATCATCTTCTGATTCCACCTCGGGATCTGGAT
	CTGACTCGGACTCTGTAATTTCCGTTACGGATTGGCAAATCTTATCATTGGTCGGTGTTTGGTCTTGCTT
	TGTGACTTTGATAATAACATCGATTCCCATATGATGTTTGTTTTCTTCTTCCGTACACGAGGAGGAGGAT
	GAGGATGATTGCTGAAGACTGGCAGGCACATGCATGCCAGGACGATATATTGTTTCATGATTGCTATTGA
	TTGAGTACTGTTCTTTATGATTCTACTTCCTTACCGTGCAATAAATTAGAATATATTTTCTACTTTTACG
	AGAAATTAATTATTGTATTTATTATTTATGGGTGAAAAACTTACTATAAAAAGCGGGTGGGTTTGGAATT
	AGTGATCAGTTTATGTATATCGCAACTACCGGGCATATGGCTATCGACATCGAGAACATTACCCACATGA
	TAAGAGATTGTATCAGTTTCGTAGTCTTGAGTATTGGTATTACTATATAGTATATAGATGTCGACGCTAG
	ATAGACAGTCTCCGAATGCGGCATGATACCGTCATCATTCTTTGCTTTCGTTAACTGTTTGGAGGAAAAA
	TTTTTGTTATTGCATTTAATCTCGAAATTCAGAGTGCACACCTTTCTCCTGTAAAGAAACCTGAAGTTGC
	TACCTTATTAAGGACGGAGAAGTATTCCTCACGAAATACGGGATTACAGTCTTTATGATTCATAGTAATA
	GTTAGTTCCGACGTTGAGATGGATTCGCTGAGACCGGTAGTGGTCGTTAACTGGATACAGATTAATTTCC
	ACATCGATATAGTTAAAGGTATTACTGGGTACGGGTTCGCATTTATCTGCGGAAGAGACGGTGTGAGAAT
	ATGTTCCGAGACCACACGGAGAACAGATGACGTCTCCGGATACTCCGTATCCTATTCCACATTTTGTTTG
	GGAAACACATGCCTTGCATCCGGATGATCCTTTGAGAAGACAATAATATCCGGGAGAGCATTCACAGATT
	CTATTGTGAGTCGTGTTACACGGTCGCGTCTTCCGTTACAACTTAGACAAGCGGGTAAATGATTATTGCG
	AGATGTGAAGGTACCCGAACCACACGGCGTACATTGTGTGTTAGTCTTGCTATCGCATAATCTGGAAGCG
	TATGTTCCCGGACACAAATTATGGCGTTTGTATTCGTTGTCTTTACACTTTCCATCGGATGGTGCATGCG
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	TTAATCCATCTGTATTAAAGATATTGTTACACCACGGCATGCGTAACTTTGATAGCAAGGATGAAAAAGG
	ACACCACTATCAATCGATAACAAGATCTTTGATATACTAACGGACACCATTGATGACTTTAGTAAATCAT
	CCGATCTATTGCTGTGTTATCTTAGATATAAATTCAATGGGAGCTTAAACTATTACGTTCTGTACAAAGG
	ATCCGACGAGGATGAACTCACTTCTCTTCATTACTACTGTAAACACATATCCACGTTCTACAAAAGCAAT
	TATTACAAGTTAAGTCACACTAAGATGCGAGCCGAGAAGCGATTCATCTACGCGATAATAGATTATGGAG
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	TCGCGGAACTACATAGTAAATTCTGTTTAAACGAGGACTATAAAAGTGTATTTATGAAAAATATATCATT
	CGATAAGATAGATTCCATCATCGTGACATAAGTCGCCTTAAAGAGATTCGAATCTCCGACACCGACCTGT
	ATACGGTATCACAGCTATCTTAAAGCCATACATTCAGACAGTCACATTTCATTTCCCATGTACGACGATC
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	TGCGAACCCGTACCCAGTAATACCTTTAACTATATCGATGTGGAAATTAATCTGTATCCAGTTAACGACC
	ACTACCGGTCTCAGCGAATCCATCTCAACGTCGGAACTAACTATTACTATGAATCATAAAGACTGTAATC
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	CTTCACTCATCGATTCAACAAAACTCAAAGCGTGTGTCTGAATCGATAACTCTATTCATCTGAAATTGGA
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	TTTTATTCTCTTTCTCTCTTTGATGGTCTCATAAAAAAAGTTTTACAAAAATATTTTTATTCTCTTTCTC TCTTTGATGGTCTCATAAAAAAAGTTTTACAAAAATATTTTTATTCTCTTTCTCTCTTTGATGGTCTCAT AAAAAAAGTTTTACAAAAATATTTTTATTCTCTTTCTCTCTTTGATGGTCTCATAAAAAAAGTTTTACAA AAATATTTTTATTCTCTTTCTCTCTTTGATGGTCTCATAAAAAATATTAAACCTCTTTCTGATGGTGTCA CTAAAATATTTTTATTCTCATTCTCATTTTCTCTTTCTCTCTTCAATGGAGTCATAAAATATTTTTATTC TCTTTCTCTCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGTGTCACTAAAATATTTTTAT TCTCATTCTCATTTTCTCTTTCTCTCTTCAATGGAGTCATAAAATATTTTTATTCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAAAAAGTTTTATCTCTTTCTCTCTTCGA TGGTCTCACTAAAATATTTTTTATTCTCTTTCTGATGCATCAACTATTTCTTAAACAATAACGTCCAACA ACATATACTCGTCGAGCTTATCAACATCCCCTATGCCCATCTAGGTTACCAGACAATTGTATATCATAAA ATAATGTTTATAATTTACACGTTAAAATCATATAATAAAACGTAGATCGTATAATATTTTTTGGTATATA AATGATCTAGTAAAATCCATGTAGGGGATACTGCTCACATTTTTTCTTTGGTACAAAATTTCACACAAGT TTTTATACAGACAAATTCTTGTCCATATATTTTAAAACATTGACTTTTGTACTAAGAAAAATATCTAGAT CAACTATCTCTTTCTCTTTCTCTCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGT AAAAAAGTTTTATCTCTTTCTCTCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGT AAAAAAGTTTTATCTCTTTCTCTCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGT AAAAAAGTTTTATCTCTTTCTCTCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGT AAAAAAGTTTTATCTCTTTCTCTCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGT AAAAAAGTTTTATCTCTTTCTCTCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGT AAAAAAGTTTTATCTCTTTCTCCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTA AAAAAGTTTTATCTCTTTCTCTCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTA AAAAAGTTTTATCTCTTTCTCTCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTA AAAAAGTTTTATCTCTTTCTCCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAA AAAAGTTTTATCTCTTTCTCCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAA AAAGTTTTATCTCTTTCTCTCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGAGTCGTAAA AAAGTTTTATCTCTTTCTCTCTTCGATGGTCTCACAAAAATATTAAACCTCTTTCTGATGGTCTCTATAA AGCGATCGATCTTTCTTACACTCTAGAGTTTCCTACAGTCATGGGTCACACATTTTTTTCTAGACACTAA ATAAAATTAGTAAAATTAAATTAATTATAAAATTATATATATAATTTACTAACTTTAGTTAGATAAATTA ATAATATATAAGTTTTAGTACATTAATATTATATTTTAAAT

[0062] In some embodiments, the oncolytic virus (e.g., a producer virus, engineered producer virus as described herein) can comprises various exogenous nucleic acids, alone, or in combination with viral gene modifications, such as mutation or deletion (complete or partial deletion) or viral genes. Non-limiting examples of viral genes can include: B5R, A52R (VACWR178), F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, C12L, TK, and any combinations thereof. Non-limiting examples of exogenous nucleic acids can include nucleic acid sequences coding for: CXCR4, CCR2, PH-

20, HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, a metabolic modulating protein, a cytokine, a fusion protein comprising any combinations of the above, and a functional domain or fragment or variant thereof, or any combinations thereof

[0063] Hyaluronan (HA) is an important structural element of ECM and a high molecular weight linear glycosaminoglycan consisting of repeating disaccharide units. It can be distributed widely throughout connective, epithelial, and neural tissues, and its expression level can be significantly elevated in many types of tumors. Hyaluronidases are a family of enzymes that catalyze the degradation of HA. There are at least five functional hyaluronidases identified so far in human: HYAL1, HYAL2, HYAL3, HYAL4 and HYAL5 (also known as PH-20 or SPAM1), among which PH-20 is the only one known so far to be functional at relatively neutral pH. In some embodiments of the present disclosure, combining hyaluronidase with other tumor-targeting therapeutic agents (such as transgenes, also referred to herein as exogenous nucleic acid) can promote the therapeutic effect of the modified oncolytic viruses that can be used as producer viruses as described herein, at least by diminishing the ECM and enhancing the transportation of the therapeutic agent inside and between the tumors.

[0064] Some embodiments herein disclose a modified oncolytic virus that can be used as a producer virus as described herein, that can comprise an exogenous nucleic acid coding for a membrane associated protein that is capable of degrading hyaluronan, such as a hyaluronidase. It should be noted that the term "hyaluronidase" as used herein can refer to any enzyme or a fragment thereof that catalyzes the degradation of HA in a tumor, including, but not limited to, PH-20 and its homologs from other species, as well as other engineered/design proteins with similar enzymatic function. As used herein, hyaluronidase can refer to a class of hyaluronan degrading enzymes.

[0065] In some embodiments, the modified oncolytic virus that can be used as a producer virus as described herein can comprises an exogenous nucleic acid that can code for a chemokine receptor that is a chimeric protein. At least part of its extracellular domain can be from a chemokine receptor that promotes the tumor-targeted delivery of the virus, and at least part of its intracellular domain can be from a chemokine receptor that promotes the tumor-specific replication, inhibits immunosuppressive activity, or conveys some other beneficial effects, or vice versa. For instance, the modified oncolytic virus can comprise a nucleic acid that codes for a protein having an intracellular GTPase domain of CCR5, and an extracellular chemokine-binding domain of CXCR4 or CCR2. In some case, by combining domains with different functionalities one may achieve further improvement in therapeutic performance of

the modified oncolytic virus. It is one embodiment of this disclosure that the modified oncolytic virus can comprise exogenous nucleic acids that can code for at least one chemokine receptor. In some cases, the modified oncolytic virus can comprise exogenous nucleic acids that can code for two or more different chemokine receptors, which may be expressed simultaneously by the virus. Exemplary chemokine receptors that can be expressed simultaneously from the modified oncolytic viruses described herein can include CXCR4 and CCR2. In modified oncolytic viruses expressing more than one chemokine receptors, a combinatorial or synergistic effect against tumor cells may be achieved as to the therapeutic application of the oncolytic virus.

[0066] In certain embodiments, the modified oncolytic virus comprises an exogenous CXCR4-expressing nucleic acid. In certain embodiments, the modified oncolytic virus comprises an exogenous CCR2-expressing nucleic acid. Certain embodiments disclose a modified oncolytic virus comprising an exogenous nucleic acid that codes for both CXCR4 and CCR2, and both chemokines are expressed form the same virus. Under certain circumstances, CXCL12 and/or CCL2 typically expressed in the tumor microenvironment may attract the CXCR4 and/or CCR2-expressing lymphocytes or other migrating cells that are infected by the modified oncolytic virus, thereby enhancing the tumor-targeted delivery of the modified oncolytic virus.

[0067] In certain embodiments, producer viruses described herein can comprise one or more exogenous nucleic acid sequences, alternatively referred to as transgenes, which can generate mRNAs coding for an agent that can modulate the activity of STAT3 and as a result can also modulate the activation of genes regulated by STAT3. Thus, certain examples provided herein provide oncolytic vaccinia viruses containing exogenous nucleic acid sequences that can encode an agent that can modulate STAT-3 mediated gene-activation. The phrase "modulates STAT 3 -mediated gene activation," as used herein, can refer to a process wherein STAT3 activity is modulated and as a consequence the activation of one or more genes that are regulated by STAT3 is also modulated.

[0068] In certain embodiments, the agent that can modulate STAT3-mediated gene activation can be a protein or a fragment thereof. In certain embodiments, the protein or the fragment thereof can inhibit, reduce, or minimize STAT3 activity and STAT3-mediated gene activation. A protein or a fragment thereof that inhibits, reduces and/or minimizes STAT3 activity and STAT3 -mediated gene activation can, for example, block the binding of STAT3 to a DNA binding sequence in the promoter regions of STAT3 responsive genes. In additional examples, the protein or a fragment thereof that inhibits, reduces, or minimizes

STAT3 activity and STAT3 -mediated gene activation can directly bind the STAT3 protein, for example, at the SH2 domain. In certain embodiments, a protein that inhibits, reduces and/or minimizes STAT3 activity blocks, prevents, reduces and/or minimizes the phosphorylation of STAT3 and/or dephosphorylates STAT3. In certain non-limiting embodiments, the proteins that modulate STAT3 activity can include phosphotyrosine phosphatases (PTPs), protein inhibitor of activated STAT (PIAS, e.g., PIAS3) and suppressor of cytokine signaling (SOCS) proteins (e.g., SOC3).

[0069] For example, in some aspects, a vaccinia virus genome can comprise an exogenous *env*, packaging construct, and a transfer construct of a lentivirus. Exogenous sequences can be inserted at any portion of a vaccinia virus genome. In an aspect, an exogenous sequence can be inserted in proximity to a 5' or 3' end. In an aspect, an exogenous sequence is inserted adjacent to another exogenous sequence. In an aspect, an exogenous sequence is inserted from about 0.5 kb to about 1000 kb away from the next nearest exogenous sequence. In some instances, the exogenous sequences coding for the lentivirus proteins can be under the control of early vaccinia virus promoters. In some embodiments, the producer virus can be an oncolytic vaccinia virus which expresses a bacteriophage regulatory sequence, such as a bacteriophage promoter, a bacteriophage termination sequence, or combinations thereof. Bacteriophage promoters or termination sequences can be from T3 bacteriophage, T7 bacteriophage and SP6 bacteriophage. The bacteriophage regulatory sequences can be cloned using standard methods for cloning and manipulating. In some embodiments, the producer virus can be an oncolytic vaccinia virus which expresses a bacteriophage polymerase. The term "bacteriophage polymerase" can refer to any bacteriophage polymerase, including those compatible with T3 bacteriophage, T7 bacteriophage and SP6 bacteriophage promoters. In some embodiments, the producer virus can be an oncolytic vaccinia virus which expresses a bacteriophage polymerase and the bacteriophage polymerase can be cloned using standard methods of cloning and manipulating.

Env element

[0070] In some aspects, an exogenous sequence can be an *env* element. In some aspects, an *env* element can be from a *vsvg*-containing *env* construct. In some aspects, a *vsvg*-containing *env* construct may recombine with the vaccinia virus genome at a point between the *vacwr032* (*k1l*) and *vacwr033* (*k2l*) genes. In an aspect, a *vsvg*-containing *env* construct can be designed to recombine with a vaccinia virus genome at a point between the *vacwr032* (*k1l*) and *vacwr033* (*k2l*) genes. This genomic location can offer a few advantages: (1) it provides adequate space between genes, (2) an identifiable promoter could be found for *vacwr032*, and

(3) this positions the construct at the 5' end of the genome which is the furthest away from any other exogenous sequences that can disrupt an endogenous vaccinia virus genome. In an aspect, the *vsvg* gene and its promoter can be positioned 3' to 5' to match the direction of the adjacent VV genes. In some cases, an *env* is inserted at position bp 26041 of a vaccinia genome. In an aspect, an envelope construct can be inserted between genes vacwr032 and vacwr033 of an oncolytic vaccinia virus. In an aspect, an envelope construct can be inserted between genes vacwr032 or a portion thereof and vacwr033 or a portion thereof of the oncolytic vaccinia virus.

[0071] In an aspect, an *env* element comprises a sequence with at least about 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 2. In an aspect, an *env* element comprises a vsvg sequence with at least about 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 3.

Packaging construct

[0072] In an aspect, an exogenous sequence can be a packaging construct sequence. In an aspect, a packaging construct can be designed to recombine with a vaccinia virus genome to replace the coding sequence for a gene, for example *vacwr094* (*j2r*) between the *vacwr093* (*j1r*) and *vacwr033* (*j3r*) genes. In an aspect, a genomic disruption caused by an insertion of an exogenous sequence can be performed. In an aspect, a genomic disruption can attenuate a function of a virus. For example, a packaging construct can be designed to recombine with a vaccinia virus genome to replace or disrupt the coding sequence for a gene because a disruption, for example a*j2r*-deletion, creates an attenuated virus that preferentially infects tumor cells. Also, this disruption/insertion positions the exogenous packaging construct near the center of the vaccinia virus genome which is the relatively distant from the other exogenous sequences, such as *env*. In some cases, a packaging construct is inserted or deleted at position bp 80724-81257 of a vaccinia virus genome. In an aspect, a packaging construct can be inserted between genes vacwr093 and vacwr095 of an oncolytic vaccinia virus. In an aspect, a genome of an oncolytic vaccinia virus comprises a deletion of the vacwr094 gene or portion thereof. In an aspect, a packaging construct can be inserted into the location of the vacwr094 gene or portion thereof. In an aspect, a packaging construct can be inserted between genes vacwr093 or a portion thereof and vacwr095 or a portion thereof of an oncolytic vaccinia virus. In an aspect, a genome of an oncolytic vaccinia virus comprises a deletion of a vacwr094 gene or portion thereof. In an aspect, a packaging construct can be inserted into the location of the vacwr094 gene or portion thereof.

[0073] In an aspect, an packaging element comprises a sequence with at least about 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 4. In an aspect, an packaging element comprises a *gag/pol* sequence with at least about 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 5. In an aspect, the nucleic acid that codes for the lentiviral structural protein comprises a nucleotide sequence as set forth in SEQ ID No. 4, or a nucleotide sequence that is at least about 70% to at least about 99% identical to the sequence set forth as SEQ ID No. 5

Transfer construct

[0074] In an aspect, an exogenous sequence can be a transfer construct sequence. In an aspect, vaccinia virus late genes may not be transcribed into single gene-containing mRNA, but instead from large 10-20 kb mRNA that may have been initiated using promoters several genes away before terminating. In an aspect, a disrupted vaccinia virus genome may comprise a transfer construct between *vacwr205* and *vacwr206*, a region with no late gene transcripts near the far 3' end of the VV genome. In some cases, a transfer construct is inserted at bp 183645 of a vaccinia virus genome. In an aspect, a transfer construct can be inserted between genes vacwr205 or portion thereof and vacwr206 or portion thereof of an oncolytic vaccinia virus.

[0075] In an aspect, a vaccinia virus provided herein can comprise at least 1 promoter. In an aspect, a vaccinia virus provided herein can comprise from about 1 promoter to about 3 promoters. In an aspect, a vaccinia virus provided herein can comprise from about 1, 2, 3, 4, 5, 6, 7, 8, 9, or up to about 10 promoters. In an aspect, a promoter can be an early promoter. In an aspect, a promoter can be an early/late promoter or a later promoter. In some cases, the transfer construct can further comprise a gene that codes for a self-cleaving RNA ribozyme, such as a hepatitis delta virus ribozyme or a hammerhead ribozyme. Without being bound by any particular theory, it is contemplated that when a self-cleaving RNA sequences are transcribed, they can cleave themselves rapidly and during transcription. Thus, the system may not have to rely on early expression to terminate the RNA as they would likely rapidly cleave themselves at the correct spot. As such, using a late promoter (*e.g.*, a vaccinia virus late promoter) can be advantageous, because now all of the retroviral (*e.g.*, lentiviral) components can be expressed with the late promoters, such as the VV late promoters, which have magnitudes higher expression, and can produce markedly more retrovirus (e.g., lentivirus). The gene that codes for the self-cleaving ribozyme, can, in some instances be positioned 3' of the termination sequence (*e.g.*, U5NU in Fig. 4) of the transfer construct.

[0076] In an aspect, a transfer construct can be inserted between genes vacwr205 or a portion thereof and vacwr206 or a portion thereof of the oncolytic vaccinia virus. In an aspect, a transfer element comprises sequence with at least about 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 6.

Lentivirus

[0077] In an aspect, provided herein can be an oncolytic virus, such as a vaccinia virus, that produces from its genome a non-replicating lentivirus or portion thereof. In some embodiments, a non-replicating lentivirus comprises a transgene that codes for a non-lentiviral protein or functional fragment thereof. In an aspect, a non-replicating lentivirus is capable of infecting and transducing both dividing and non-dividing cells in a target population of mammalian cells. In an aspect, mammalian cells can be human.

[0078] In an aspect, provided herein can be a second-generation lentivirus. A second-generation lentivirus may comprise a transfer plasmid that may utilize a tat element for packaging. In an aspect, a second-generation lentivirus may comprise packaging elements in a single plasmid, for example: *Gag*, *pol*, *rev*, and tat. In an aspect, a second-generation lentivirus may comprise an *env* element that can encode for VSV-G. A second-generation lentivirus may be a 3-plasmid system. In an aspect, a second-generation lentivirus may utilize a wild type LTR. Second generation lentiviruses can be replication incompetent and may utilize 3 plasmids to encode various HIV genes.

[0079] In an aspect, provided herein can be a third-generation lentivirus. A third-generation lentivirus may utilize a second generation or third generation packaging system to package a transfer element. In an aspect, a third-generation lentivirus utilizes at most 2 plasmids to package, for example one plasmid encoding *gag* and *pol* and a second plasmid encoding *rev*. In an aspect, a third-generation lentivirus may comprise an *env* element that can encode for VSV-G. In an aspect, a third-generation lentivirus uses up to 4 plasmids, and may eliminate the use of a tat element. In an aspect, a third-generation lentivirus may be safer than a second-generation lentivirus. A third-generation lentivirus may comprise a hybrid LTR viral promoter, for example a 5'LTR can be deleted or partially deleted and can be fused to an exogenous promoter, for example a vaccinia virus, CMV or RSV promoter.

[0080] In some cases, provided herein can be a lentivirus or a portion thereof. In some cases, a lentivirus can be derived from an HIV-1 virus. In some aspects, a lentivirus can be derived from a non-HIV-1 virus, for example: HIV-2, simian immunodeficiency virus, feline immunodeficiency virus, bovine immunodeficiency virus, or caprine arthritis-encephalitis virus, equine infectious anemia virus (EIAV), and any combination thereof.

[0081] In some cases, provided herein can be a lentivirus from an HIV-1 virus. In some aspects, multiple plasmids coding for different components of an HIV-1 virus can be delivered on multiple plasmids. A lentiviral vector system can comprise at least two plasmids that comprise portions of the lentivirus. In some aspects, at least 2, 3, 4, 5, 6, 7, or up to about 8 plasmids can comprise portions of the lentivirus. In some aspects, an LV vector can be a 3-plasmid system. In some cases, a 3-plasmid system comprises two helper plasmids coding for *gag-pol* and the *env* functions as well as the TV plasmid. In some aspects, an LV vector can be a 4-plasmid system. 4 plasmid LV systems can comprise accessory genes of HIV-1 (for example: vif, vpr, vpu, and nef), may be present only in a first generation of LV. In some aspects, they have been removed because they may not be necessary. Similarly, the regulatory tat gene, present in the second-generation LV, has been eliminated in some cases because its transacting function is dispensable as the U3 promoter of the 5' long terminal repeat (LTR) in the TV has been replaced by a constitutively active promoter sequence. In some aspects, a vaccinia virus may be absent a gene from a foreign virus, such as an HIV-1 *rev* gene. In some aspects, a vector provided herein may contain a hybrid 5' LTR region. A hybrid 5' LTR region may contain at least one component from a vaccinia virus and at least one component from an HIV-1. In some aspects, a component from an HIV-1 is a 5' LTR. In some aspects, a component from an HIV-1 is a 3' LTR. A hybrid construct may be absent tat. In some aspects, a hybrid construct may utilize a vaccinia virus transcription factor in place of tat.

[0082] In some embodiments, a constitutively active promoter sequence can be any one of: cytomegalovirus (promoter) or Rous Sarcoma Virus (promoter). In some cases, an optional enhancer or an inducible/repressible promoter sequence, such as 7tetO may be included (pRRL (lentivirus transfer vector construct containing chimeric Rous sarcoma virus (RSV)-HIV 5' LTRs) design or the pCCL design ((CMV)-HIV 5' LTR). These modifications can lead to a LV vector system with the helper functions based on the use of *gag-pol* (encoding for the structural proteins and viral enzymes) and *rev* (encoding for a post-transcriptional regulator) derived from HIV-1 and *env*. In some aspects, a vector provided herein is absent *rev*. In some aspects, LV vectors can be pseudotyped with different heterologous envelope glycoproteins. In some aspects, a glycoprotein of the vesicular stomatitis virus (VSV-g) envelope can be utilized due to improved stability during downstream processing as well as its large transduction spectrum. In some aspects, a TV plasmid is genetic material transferred to the target cells and can comprise the LV backbone containing the transgene expression cassette flanked by cis-acting elements required for encapsidation, reverse transcription, and

integration. In some aspects, a transfected cell comprises at least a portion of a TV plasmid after infection. In some aspects, provided herein can be a self-inactivating (SIN)-LV vector. A SIN-LV vector comprises a deletion at the U3 element of the 2'LTR. In some aspects, a SIN-LV vector loses the transcriptional capacity of the viral LTR once transferred to the target cells; this can minimize the risk of emergence of replication competent recombinants and avoiding problems linked to promoter interference. In some aspects, provided herein can be a third-generation plasmid system in which tat has been removed from the system. In some aspects, a third-generation vector may replace the activity of the HIV-1 protein TAT by using a chimeric 5' LTR fused to a heterologous promoter.

[0083] In some cases, the termination of vaccinia virus early gene transcription can utilize the termination sequence UUUUUNU (U5NU) (SEQ ID NO: 7) to attract vaccinia virus-specific factors to halt transcription and generate poly (A) tails to protect viral mRNA. In an aspect, a vector provided herein may comprise the ATTTTTAT (SEQ ID NO: 8) sequence adjacent to the desired terminal region of a construct. In some embodiments, the termination sequence comprises a nucleotide sequence with percent identity from about 60%, 70%, 80%, 90%, 95%, or up to about 100% to a sequence as set forth in SEQ ID No. 7.

[0084] In certain embodiments, the retroviral vectors provided herein can be replication defective, that is, they are unable to replicate autonomously in a target cell. In some aspects, a replication defective virus is a minimal virus, such that it retains only the sequences of its genome which are necessary for target cell recognition and encapsidating the viral genome. In some cases, a replication defective virus is not infective after introduction into a cell. Use of replication defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Thus, a specific tissue can be specifically targeted.

[0085] In an aspect, a lentivirus construct provided herein, for expression from a producer oncolytic virus, may comprise a promoter. In an aspect, expression of a lentiviral element may be expressed early. In an aspect, expression of a lentiviral element may be robust. For example, a vaccinia virus may generate short, single gene mRNA with poly (A) tails during an early phase of infection, for example within 2 hr. post-infection. After that period, viral mRNA from the intermediate and late phases of infection may not be specifically terminated or polyadenylated. To form functional lentivirus transfer RNA, transcription can be initiated and terminated at specific sites adjacent to lentiviral long terminal repeats (LTRs), therefore, lentiviral elements may be expressed robustly in the first 2 hr. post vaccinia virus infection. Therefore, in some cases a vector provided herein can have strong early promoters for each

element and fused a strong early promoter to a 5' LTR of the transfer construct. In an aspect, there are from about 1-10 viral elements with preceding strong early promoters. In an aspect, there are from about 1-5 viral elements with preceding strong early promoters. In an aspect, there are from about 1-3 viral elements with preceding strong early promoters. In some instances, the components of the lentiviral construct can be under the control of early/late, or late viral promoters. For example, the packaging construct, the transfer construct, and the envelope construct each independently comprises an early/late viral promoter or a late viral promoter.

[0086] Promoters are sequences of nucleic acid that control the binding of RNA polymerase and transcription factors, and can have a major effect on the efficiency of gene transcription, where a gene may be expressed in the cell, and/or what cell types a gene may be expressed in. Non limiting examples of promoters include a vaccinia virus promoter, cytomegalovirus (CMV) promoter, an elongation factor 1 alpha (EF1α) promoter, a simian vacuolating virus (SV40) promoter, a phosphoglycerate kinase (PGK1) promoter, a ubiquitin C (Ubc) promoter, a human beta actin promoter, a CAG promoter, a Tetracycline response element (TRE) promoter, a UAS promoter, an Actin 5c (Ac5) promoter, a polyhedron promoter, Ca2+/calmodulin-dependent protein kinase II (CaMKIIa) promoter, a GAL1 promoter, a GAL 10 promoter, a TEF1 promoter, a glyceraldehyde 3-phosphage dehydrogenase (GDS) promoter, an ADH1 promoter, a CaMV35S promoter, a Ubi promoter, a human polymerase III RNA (H1) promoter, a U6 promoter, or a combination thereof.

[0087] In some embodiments, the disclosure provides a method of eliciting an antitumor immune response in a subject presenting with, or at risk of developing a cancer. In some aspects, a cancer can be metastatic. In an aspect, an oncolytic virus (e.g., HSV, vaccinia virus, or adenovirus), a retrovirus produced from the oncolytic virus, or both can express a therapeutic protein or a diagnostic protein. In an aspect, a therapeutic protein can be a PD-1 binding agent, such as a single chain anti-PD-1 antibody, that antagonizes the activity of PD-1. In other embodiments, the therapeutic protein an agent that antagonizes the binding of the PD-1 ligands to the receptor, e.g., anti-PD-L1 and/or PD-L2 antibodies, PD-L1 and/or PD-L2 decoys, or a soluble PD-1 receptor. In an aspect, PD-1 blockade may also stimulate the anti-tumor immune response by blocking the inactivation of T-cells (CTLs and helper) and B-cells thereby leading to increased cancer killing.

Packaging cell lines

[0088] In an aspect, provided herein can be a packaging cell line. The production of replication defective virus can be accomplished through trans-complementation in which

packaging cells may be cotransfected with multiple plasmids (for example from about 2, 3, 4, 5 or more plasmids) that together express all of the viral proteins necessary to generate infectious particles, as well as the nucleic acid sequence of interest that will be packaged within them for delivery, for example a viral sequences. While many lentiviral vector systems are based on transduction of two helper plasmids (second generation) with the transfer plasmid, some newer systems (third generation) have the packaging and envelope constructs on three plasmids which are combined with the transfer plasmid and utilized to transfect a packaging cell line. In an aspect, provided herein can be a packaging cell line for use with a retrovirus. In an aspect, a first-generation retroviral packaging cell line can be utilized. First generation retroviral packaging cell lines can be derived from mouse 3T3 cell. In an aspect, a second-generation retroviral vector packaging cell line can be utilized. Non-limiting examples of second-generation retroviral vector packaging cell lines include: human cells like HEK293 (embryonic kidney), TE671 (rhabdomyosarcoma) and HT1080 (fibrosarcoma). Third generation packaging cell lines may be absent unnecessary viral sequences present in second generation packaging plasmids as well as in transfer vectors have been deleted to improve safety.

Non-lentiviral proteins

Therapeutic protein or portion thereof

[0089] In some embodiments, a non-lentiviral protein can be a therapeutic protein or portion thereof. In an aspect, a therapeutic protein or portion thereof can be any one of: immune checkpoint modulator, antibody or portion thereof, Fc fusion protein, anticoagulant, blood factor, bone morphogenetic protein, immunosuppressive agents, immunostimulatory agents, enzyme, growth factor, hormone, interferon, interleukin, thrombolytic, anti-angiogenic, chemotherapeutic, antibiotic, antifungal, antiviral, and any combination thereof.

[0090] In an aspect, a therapeutic protein or portion thereof can be an immune checkpoint modulator. In an aspect, an immune checkpoint modulator can inhibit an immune checkpoint. In an aspect, an immune checkpoint modulator can activate an immune checkpoint. An immune checkpoint inhibitor can be a drug that blocks a protein made by some types of immune system cells, such as T cells, and some cancer cells. These immune checkpoint proteins help keep immune responses in check and can keep T cells from killing cancer cells. When these proteins are inhibited, the “brakes” on the immune system are released and T cells are able to kill cancer cells. Examples of checkpoint proteins found on T cells or cancer cells include but are not limited to: PD-1/PD-L1 and CTLA-4/B7-1/B7-2. In an aspect, an immune checkpoint inhibitor can be delivered by a virus provided herein.

[0091] There are a variety of immune checkpoints that can be targeted by an immune checkpoint modulator and/or any therapeutic protein, **Table 2**.

[0092] **Table 2: Genes coding for immune checkpoints that are targeted by immune checkpoint modulators**

Gene Symbol	Abbreviation	Name	NCBI number (GRCh38.p2) *AC010327.8 ** GRCh38.p7	Location in genome
ADORA 2A	A2aR; RDC8; ADORA2	adenosineA2a receptor	135	22q11.23
CD276	B7H3; B7-H3; B7RP-2; 4Ig-B7-H3	CD276 molecule	80381	15q23-q24
VTCN1	B7X; B7H4; B7S1; B7-H4; B7h.5; VCTN1; PRO1291	V-set domain containing T cell activation inhibitor 1	79679	1p13.1
BTLA	BTLA1; CD272	B and T lymphocyte associated	151888	3q13.2
CTLA4	GSE; GRD4; ALPS5; CD152; CTLA-4; IDDM12; CELIAC3	cytotoxic T-lymphocyte-associated protein 4	1493	2q33
IDO1	IDO; INDO; IDO-1	indoleamine 2,3-dioxygenase 1	3620	8p12-p11
KIR3DL 1	KIR; NKB1; NKAT3; NKB1B; NKAT-3; CD158E1; KIR3DL2; KIR3DL1/S1	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 1	3811	19q13.4
LAG3	LAG3;CD223	lymphocyte-activation gene 3	3902	12p13.32
PDCD1	PD1; PD-1; CD279; SLEB2; hPD-1; hPD-l; hSLE1	programmed cell death 1	5133	2q37.3
HAVCR 2	TIM3; CD366; KIM-3; TIMD3; Tim-3; TIMD-3; HAVcr-2	hepatitis A virus cellular receptor 2	84868	5q33.3
VISTA	C10orf54, differentiation of ESC-1 (Dies1); platelet receptor Gi24 precursor; PD1 homolog (PD1H) B7H5; GI24; B7-H5; SISP1; PP2135	V-domain immunoglobulin suppressor of T-cell activation	64115	10q22.1
CD244	2B4; 2B4; NAIL; Nmrk; NKR2B4; SLAMF4	CD244 molecule, natural killer cell receptor 2B4	51744	1q23.3

CISH	CIS; G18; SOCS; CIS-1; BACTS2	Cytokine Inducible SH2-Containing protein	1154	3p21.3
HPRT1	HPRT; HGPRT	hypoxanthine phosphoribosyltransferase 1	3251	Xq26.1
AAV*S1	AAV	adeno-associated virus integration site 1	14	19q13
CCR5	CKR5; CCR-5; CD195; CKR-5; CCCKR5; CMKBR5; IDDM22; CC-CKR-5	chemokine (C-C motif) receptor 5 (gene/pseudogene)	1234	3p21.31
CD160	NK1; BY55; NK28	CD160 molecule	11126	1q21.1
TIGIT	VSIG9; VSTM3; WUCAM	T-cell immunoreceptor with Ig and ITIM domains	201633	3q13.31
CD96	TACTILE	CD96 molecule	10225	3q13.13-q13.2
CRTAM	CD355	cytotoxic and regulatory T-cell molecule	56253	11q24.1
LAIR1	CD305; LAIR-1	leukocyte associated immunoglobulin like receptor 1	3903	19q13.4
SIGLEC 7	p75; QA79; AIRM1; CD328; CDw328; D-siglec; SIGLEC-7; SIGLECP2; SIGLEC19P; p75/AIRM1	sialic acid binding Ig like lectin 7	27036	19q13.3
SIGLEC 9	CD329; CDw329; FOAP-9; siglec-9; OBBP-LIKE	sialic acid binding Ig like lectin 9	27180	19q13.41
TNFRSF 10B	DR5; CD262; KILLER; TRICK2; TRICKB; ZTNFR9; TRAILR2; TRICK2A; TRICK2B; TRAIL-R2; KILLER/DR5	tumor necrosis factor receptor superfamily member 10b	8795	8p22-p21
TNFRSF 10A	DR4; APO2; CD261; TRAILR1; TRAILR-1	tumor necrosis factor receptor superfamily member 10a	8797	8p21
CASP8	CAP4; MACH; MCH5; FLICE; ALPS2B; Casp-8	caspase 8	841	2q33-q34
CASP10	MCH4; ALPS2;	caspase 10	843	2q33-q34

	FLICE2			
CASP3	CPP32; SCA-1; CPP32B	caspase 3	836	4q34
CASP6	MCH2	caspase 6	839	4q25
CASP7	MCH3; CMH-1; LICE2; CASP-7; ICE-LAP3	caspase 7	840	10q25
FADD	GIG3; MORT1	Fas associated via death domain	8772	11q13.3
FAS	APT1; CD95; FAS1; APO-1; FASTM; ALPS1A; TNFRSF6	Fas cell surface death receptor	355	10q24.1
TGFBRII	AAT3; FAA3; LDS2; MFS2; RIIC; LDS1B; LDS2B; TAAD2; TGFR-2; TGFbeta-RII	transforming growth factor beta receptor II	7048	3p22
TGFBR1	AAT5; ALK5; ESS1; LDS1; MSSE; SKR4; ALK-5; LDS1A; LDS2A; TGFR-1; ACVRLK4; tbetaR-I	transforming growth factor beta receptor I	7046	9q22
SMAD2	JV18; MADH2; MADR2; JV18-1; hMAD-2; hSMAD2	SMAD family member 2	4087	18q21.1
SMAD3	LDS3; LDS1C; MADH3; JV15-2; HSPC193; HsT17436	SMAD family member 3	4088	15q22.33
SMAD4	JIP; DPC4; MADH4; MYHRS	SMAD family member 4	4089	18q21.1
SKI	SGS; SKV	SKI proto-oncogene	6497	1p36.33
SKIL	SNO; SnoA; SnoI; SnoN	SKI-like proto-oncogene	6498	3q26
TGIF1	HPE4; TGIF	TGFB induced factor homeobox 1	7050	18p11.3
IL10RA	CD210; IL10R; CD210a; CDW210A; HIL-10R; IL-10R1	interleukin 10 receptor subunit alpha	3587	11q23
IL10RB	CRFB4; CRF2-4; D21S58; D21S66; CDW210B; IL-10R2	interleukin 10 receptor subunit beta	3588	21q22.11
HMOX2	HO-2	heme oxygenase 2	3163	16p13.3
IL6R	IL6Q; gp80; CD126; IL6RA; IL6RQ; IL-6RA; IL-6R-1	interleukin 6 receptor	3570	1q21
IL6ST	CD130; GP130; CDW130; IL-6RB	interleukin 6 signal transducer	3572	5q11.2
CSK	CSK	c-src tyrosine kinase	1445	15q24.1
PAG1	CBP; PAG	phosphoprotein membrane anchor	55824	8q21.13

		with glycosphingolipid microdomains 1		
SIT1	SIT1	signaling threshold regulating transmembrane adaptor 1	27240	9p13-p12
FOXP3	JM2; AIID; IPEX; PIDX; XPID; DIETER	forkhead box P3	50943	Xp11.23
PRDM1	BLIMP1; PRDI-BF1	PR domain 1	639	6q21
BATF	SFA2; B-ATF; BATF1; SFA-2	basic leucine zipper transcription factor, ATF-like	10538	14q24.3
GUCY1A2	GC-SA2; GUC1A2	guanylate cyclase 1, soluble, alpha 2	2977	11q21-q22
GUCY1A3	GUCA3; MYMY6; GC-SA3; GUC1A3; GUCSA3; GUCY1A1	guanylate cyclase 1, soluble, alpha 3	2982	4q32.1
GUCY1B2	GUCY1B2	guanylate cyclase 1, soluble, beta 2 (pseudogene)	2974	13q14.3
GUCY1B3	GUCB3; GC-SB3; GUC1B3; GUCSB3; GUCY1B1; GC-S-beta-1	guanylate cyclase 1, soluble, beta 3	2983	4q31.3-q33
TRA	IMD7; TCRA; TCRD; TRAalpha; TRAC	T-cell receptor alpha locus	6955	14q11.2
TRB	TCRB; TRBbeta	T cell receptor beta locus	6957	7q34
EGLN1	HPH2; PHD2; SM20; ECYT3; HALAH; HPH-2; HIFPH2; ZMYND6; C1orf12; HIF-PH2	egl-9 family hypoxia-inducible factor 1	54583	1q42.1
EGLN2	EIT6; PHD1; HPH-1; HPH-3; HIFPH1; HIF-PH1	egl-9 family hypoxia-inducible factor 2	112398	19q13.2
EGLN3	PHD3; HIFPH3; HIFP4H3	egl-9 family hypoxia-inducible factor 3	112399	14q13.1
PPP1R12C**	p84; p85; LENG3; MBS85	protein phosphatase 1 regulatory subunit 12C	54776	19q13.42

[0093] The therapeutic protein, in some cases, can be a humanized anti-CD20 monoclonal antibody (*e.g.*, Gazyva), a VEGFR Fc-fusion (*e.g.*, Eylea), a CTLA-4 Fc-fusion (*e.g.*, Nulojix), a glucagon-like peptide-1 receptor agonist Fc-fusion (*e.g.*, Trulicity), VEGFR Fc-

fusion (e.g., Zaltrap), a recombinant factor IX Fc fusion (e.g., Alprolix), a recombinant factor VIII Fc-fusion (*e.g.*, Eloctate), a GLP-1 receptor agonist-albumin fusion (*e.g.*, Tanzeum), a recombinant factor IX albumin fusion (*e.g.*, Idelvion), a PEGylated IFNβ-1a (*e.g.*, Plegridy), a recombinant factor VIII PEGylated (*e.g.*, Adynovate), a humanized anti-HER2/neu conjugated to emtansine (*e.g.*, Kadcyla), a mouse/human chimeric anti-CD30 (*e.g.*, Adcetris), an anti-human epidermal growth factor receptor 2 (HER2) (*e.g.*, Perjeta), Anti-IL-6 receptor (Actemra), an anti-CD20 (*e.g.*, obinutuzumab; Gazyva), an anti-integrin a4b7 (LPAM-1) (*e.g.*, Entyvio), an anti-PD-1 (e.g., Keytruda), an anti-dabigatran (*e.g.*, Praxbind), an anti-IL-5 (*e.g.*, Nucala), an Anti-CD319 (SLAMF7) (*e.g.*, Empliciti), an anti-IL-17a (*e.g.*, Taltz), an anti-IL-5 (*e.g.*, Cinqair), an anti-PD-L1 (*e.g.*, Tecentriq), an anti-CD25 (*e.g.*, Zinbryta), an anti-CD30 (*e.g.*, Adcetris), an anti-IL-6 (*e.g.*, Sylvant), an anti-GD2 (*e.g.*, Unituxin), an anti-*Bacillus anthracis* (*e.g.*, Anthim), an anti-TNFα (*e.g.*, Inflectra), a human anti-B-cell activating factor (BAFF) (*e.g.*, belimumab), a human anti-CTLA-4 (*e.g.*, ipilimumab), a CTLA-4 Fc-fusion (*e.g.*, belatacept), humanized anti-human epidermal growth factor receptor 2 (HER2) (*e.g.*, pertuzumab), a VEGFR Fc fusion (*e.g.*, ziv-afilbercept), a G-CSF (*e.g.*, tbo-filgrastim), human anti-VEGFR2 (KDR) (*e.g.*, ramucirumab), a mouse/human chimeric anti-IL-6 (*e.g.*, siltuximab), pembrolizumab, mouse bispecific anti-CD19/anti-CD3 (*e.g.*, blintumomab), nivolumab, a parathyroid hormone, a mouse/human chimeric anti-GD2 (*e.g.*, dinutuximab), a human anti-CD38 (*e.g.*, daratumumab), a human anti-epidermal growth factor receptor (EGFR)(*e.g.*, necitumumab), humanized anti-CD319(SLAMF7) (*e.g.*, elotuzumab), atezolizumab.

[0094] In an aspect, a therapeutic protein can be a cytokine. A cytokine can be a pro-inflammatory cytokine. In an aspect, a cytokine can be: IL-2, IL-7, IL-12, IL-15, IL-21, and combinations thereof. Additionally, pro-inflammatory cytokines can also be introduced with a virus provided herein. Non-limiting examples of pro-inflammatory cytokines include interleukin 6 (IL-6), interferon alpha (IFNα), interferon beta (IFNβ), C-C motif ligand 4 (CCL4), C-C motif ligand 5 (CCL5), C-X-C motif ligand 10 (CXCL10), interleukin 1 beta (IL-1β), IL-18 and IL-33.

[0095] In an aspect, a therapeutic protein can be a growth factor. In some aspects, a growth factor can be an interleukin. Non-limiting examples of interleukins include: interleukin-2 (IL-2), insulin, IFN-γ, IL-4, IL-7, GM-CSF, IL-10, IL-21, IL-15, TGF beta, and TNF alpha or any other additives for the growth of cells, such as immune cells.

[0096] In an aspect, a therapeutic protein can be a chemotherapeutic. A chemotherapeutic agent or compound can be a chemical compound useful in the treatment of cancer. The

chemotherapeutic cancer agents that can be used in combination with the disclosed viruses include, but are not limited to, mitotic inhibitors (vinca alkaloids). These include vincristine, vinblastine, vindesine and Navelbine™ (vinorelbine, 5'-noranhydroblastine). In yet other cases, chemotherapeutic cancer agents include topoisomerase I inhibitors, such as camptothecin compounds. As used herein, "camptothecin compounds" include Camptosar™ (irinotecan HCL), Hycamtin™ (topotecan HCL) and other compounds derived from camptothecin and its analogues. Another category of chemotherapeutic cancer agents that can be used in the methods and compositions disclosed herein are podophyllotoxin derivatives, such as etoposide, teniposide and mitopodozide. The present disclosure further encompasses other chemotherapeutic cancer agents known as alkylating agents, which alkylate the genetic material in tumor cells. These include without limitation cisplatin, cyclophosphamide, nitrogen mustard, trimethylene thiophosphoramide, carmustine, busulfan, chlorambucil, belustine, uracil mustard, chlomaphazin, and dacarbazine. The disclosure encompasses antimetabolites as chemotherapeutic agents. Examples of these types of agents include cytosine arabinoside, fluorouracil, methotrexate, mercaptopurine, azathioprime, and procarbazine.

[0097] In an aspect, a therapeutic agent can be an antibiotic. In some cases, an antibiotic can be a bacterial wall targeting agent, a cell membrane targeting agent, a bacterial enzyme interfering agent, a bactericidal agent, a protein synthesis inhibitor, or a bacteriostatic agent. A bacterial wall targeting agent can be a penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems. β-Lactam antibiotics are bactericidal or bacteriostatic and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. In some cases, an antibiotic may be a protein synthesis inhibitor. A protein synthesis inhibitor can be ampicillin which acts as an irreversible inhibitor of the enzyme transpeptidase, which is needed by bacteria to make the cell wall. It inhibits the third and final stage of bacterial cell wall synthesis in binary fission, which ultimately leads to cell lysis; therefore, ampicillin is usually bacteriolytic. In some cases, a bactericidal agent can be cephalosporin or quinolone. In other cases, a bacteriostatic agent is trimethoprim, sulfamethoxazole, or pentamidine. Additional examples of antibiotics include without limitation doxorubicin, bleomycin, dactinomycin, daunorubicin, mithramycin, mitomycin, mytomycin C, and daunomycin.

[0098] In an aspect, a therapeutic agent can also be an antibody or portion thereof. An antibody can be a polyclonal antibody or a monoclonal antibody. A polyclonal antibody that can be administered can be an antilymphocyte or antithymocyte antigen. A monoclonal

antibody can be an anti-IL-2 receptor antibody, an anti-CD25 antibody, or an anti-CD3 antibody. An anti-CD20 antibody can also be used. B-cell ablative therapy such as agents that react with CD20, *e.g.*, Rituxan can also be used as immunosuppressive agents. Exemplary anti-cancer antibodies include but are not limited to: dacarbazine, azacytidine, amsacrine, melphalan, ifosfamide and mitoxantrone.

[0099] In an aspect, a therapeutic agent can also be an anti-fungal. In some cases, an antifungal agent can be from a class of polyene, azole, allylamine, or echinocandin. In some embodiments, a polyene antifungal is amphotericin B, candicidin, filipin, hamycin, natamycin, nystatin, or rimocidin. In some cases, an antifungal can be from an azole family. Azole antifungals can inhibit lanosterol 14 α-demethylase. An azole antifungal can be an imidazole such as bifonazole, butoconazole, clotrimazole, econazole, fenticonazole, isoconazole, ketoconazole, luliconazole, miconazole, omoconazole, oxiconazole, sertaconazole, sulcoazole, or tioconazole. An azole antifungal can be a triazole such as albaconazole, efinaconazole, epoxiconazole, fluconazole, isavuvonazole, itraconazole, posaconazole, propiconazole, ravuconazole, terconazole, or voriconazole. In some cases, an azole can be a thiazole such as abafungin. An antifungal can be an allylamine such as amorolfin, butenafine, naftifine, or terbinafine. An antifungal can also be an echinocandin such as anidulafungin, caspofungin, or micafungin. Additional agents that can be antifungals can be aurones, benzoic acid, ciclopirox, flucytosine, griseofulvin, haloprogin, tolnaftate, undecylenic acid, crystal violet or balsam of Peru.

Pharmaceutical Compositions

[00100] Provided herein is a pharmaceutically acceptable viral therapeutic. In an aspect, provided herein are also methods of implementing pharmaceutically acceptable viral therapeutics comprising oncolytic viruses. Compositions and methods provided herein can be used for the treatment of a disease, such as a cancer.

[00101] Cancer can include, but is not limited to, melanoma, hepatocellular carcinoma, breast cancer, lung cancer, peritoneal cancer, prostate cancer, bladder cancer, ovarian cancer, leukemia, lymphoma, renal carcinoma, pancreatic cancer, epithelial carcinoma, gastric cancer, colon carcinoma, duodenal cancer, pancreatic adenocarcinoma, mesothelioma, glioblastoma multiforme, astrocytoma, multiple myeloma, prostate carcinoma, hepatocellular carcinoma, cholangiosarcoma, pancreatic adenocarcinoma, head and neck squamous cell carcinoma, colorectal cancer, intestinal-type gastric adenocarcinoma, cervical squamous-cell carcinoma, osteosarcoma, epithelial ovarian carcinoma, acute lymphoblastic lymphoma, myeloproliferative neoplasms, and sarcoma.

[00102] In an aspect, an oncolytic virus can amplify a therapeutic agent selectively within a cancer. In an aspect, an oncolytic virus can also carry a therapeutic protein or a diagnostic protein and express the protein or a functional fragment thereof in proximity to a cancer. In an aspect, a therapeutic protein or a diagnostic protein can enhance an endogenous immune response. In an aspect, an oncolytic virus and/or a retrovirus provided herein coupled with a therapeutic protein provided herein can have greater anti-cancer effects as compared to a comparable virus absent the coupling with the therapeutic agent. In an aspect, the oncolytic virus and/or a retrovirus provided herein coupled with a therapeutic protein provided herein can have from about 1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, or up to about 10 fold greater anti-cancer effects as compared to a comparable a virus absent the coupling with the therapeutic agent. In an aspect, the oncolytic virus and/or a retrovirus provided herein coupled with a therapeutic protein provided herein can reduce a cancer by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or up to 100% more as compared to a comparable virus absent the coupling with the therapeutic agent.

[00103] In an aspect, provided herein can also be methods of reducing toxicity of cells contacted with nucleic acids, polypeptides, vectors, and plasmids provided herein. For example, a vector encoding for a virus provided herein can be polycistronic, thereby introducing multiple elements, such as a virus, in a single vector construct and reducing toxicity associated with a vector. In an aspect, a bicistronic vector coding for an oncolytic virus provided herein and a therapeutic protein provided herein can have from about 1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, or up to about 10 fold less toxicity as compared to introducing two monocistronic vectors the first one coding for the oncolytic virus provided herein and the second one coding for the therapeutic agent. In some cases, cell toxicity is reduced by about, at least about, or at most about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 15%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 82%, 85%, 88%, 90%, 92%, 95%, 97%, 98%, 99% or 100% when at least a bicistronic vector provided herein is used as compared to introducing each element alone in a dual vector system.

[00104] As described herein, viral particles provided herein, can be used to deliver a viral vector comprising a transgene comprising at least a portion of a replication defective virus into a cell *ex vivo* or *in vivo*. In some embodiments, a virus provided herein can be measured as pfu (plaque forming units). In some cases, the pfu of a viral dosage of the compositions and methods of the disclosure can be about 10^8 to about 5×10^{10} pfu. In some cases, a dosage of a virus provided herein is at least about 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8, 6×10^8, 7×10^8,

8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, 5×10^9, 6×10^9, 7×10^9, 8×10^9, 9×10^9, 1×10^{10}, 2×10^{10}, 3×10^{10}, 4×10^{10}, and 5×10^{10} pfu. In some cases, a viral of this disclosure is at most about 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, 5×10^9, 6×10^9, 7×10^9, 8×10^9, 9×10^9, 1×10^{10}, 2×10^{10}, 3×10^{10}, 4×10^{10}, 5×10^{10} , 1×10^{11}, 2×10^{11}, 3×10^{11}, 4×10^{11}, 5×10^{11}, 6×10^{11} , 7×10^{11}, 8×10^{11}, 9×10^{11} , 1×10^{12}, 2×10^{12}, 3×10^{12}, 4×10^{12}, 5×10^{12}, 6×10^{12}, 7×10^{12}, 8×10^{12}, 9×10^{12}, 1×10^{13}, 2×10^{13}, 3×10^{13}, 4×10^{13}, 5×10^{13}, 6×10^{13}, 7×10^{13}, 8×10^{13}, 9×10^{13}, 1×10^{14}, 2×10^{14}, 3×10^{14}, 4×10^{14}, 5×10^{14} , 1×10^{15}, 2×10^{15}, 3×10^{15}, 4×10^{15}, 5×10^{15}, 6×10^{15} , 7×10^{15}, 8×10^{15}, 9×10^{15} pfu. In some aspects, a viral vector of the disclosure can be measured as vector genomes. In some cases, viruses of this disclosure are 1×10^{10} to 3×10^{12} vector genomes, or 1×10^9 to 3×10^{13} vector genomes, or 1×10^8 to 3×10^{14} vector genomes, or at least about 1×10^1, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9, 1×10^{10}, 1×10^{11}, 1×10^{12}, 1×10^{13}, 1×10^{14}, 1×10^{15}, 1×10^{16}, 1×10^{17}, and 1×10^{18} vector genomes, or are 1×10^8 to 3×10^{14} vector genomes, or are at most about 1×10^1, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9, 1×10^{10}, 1×10^{11}, 1×10^{12}, 1×10^{13}, 1×10^{14}, 1×10^{15}, 1×10^{16}, 1×10^{17}, and 1×10^{18} vector genomes.

[00105] In some cases, the dosage of a virus or a viral vector of the disclosure can be measured using multiplicity of infection (MOI). In some cases, MOI can refer to the ratio, or multiple of vector or viral genomes to the cells to which the nucleic can be delivered. In some cases, the MOI can be 1×10^6 GC/mL (genome copies/mL). In some cases, the MOI can be 1×10^5 GC/mL to 1×10^7 GC/mL. In some cases, the MOI can be 1×10^4 GC/mL to 1×10^8 GC/mL. In some cases, recombinant viruses of the disclosure are at least about 1×10^1 GC/mL, 1×10^2 GC/mL, 1×10^3 GC/mL, 1×10^4 GC/mL, 1×10^5 GC/mL, 1×10^6 GC/mL, 1×10^7 GC/mL, 1×10^8 GC/mL, 1×10^9 GC/mL, 1×10^{10} GC/mL, 1×10^{11} GC/mL, 1×10^{12} GC/mL, 1×10^{13} GC/mL, 1×10^{14} GC/mL, 1×10^{15} GC/mL, 1×10^{16} GC/mL, 1×10^{17} GC/mL, and 1×10^{18} GC/mL MOI. In some cases, a virus of this disclosure are from about 1×10^8 GC/mL to about 3×10^{14} GC/mL MOI, or are at most about 1×10^1 GC/mL, 1×10^2 GC/mL, 1×10^3 GC/mL, 1×10^4 GC/mL, 1×10^5 GC/mL, 1×10^6 GC/mL, 1×10^7 GC/mL, 1×10^8 GC/mL, 1×10^9 GC/mL, 1×10^{10} GC/mL, 1×10^{11} GC/mL, 1×10^{12} GC/mL, 1×10^{13} GC/mL, 1×10^{14} GC/mL, 1×10^{15} GC/mL, 1×10^{16} GC/mL, 1×10^{17} GC/mL, and 1×10^{18} GC/mL MOI.

[00106] Vectors described herein, can be delivered by any suitable method, including transfection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion. Electroporation using, for example, the Neon® Transfection System (ThermoFisher Scientific) or the AMAXA® Nucleofector (AMAXA® Biosystems) can also be used for delivery of nucleic acids into a cell.

[00107] The transfection efficiency of cells, such as producer cells, with any of the vectors and plasmids provided herein can be or can be about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or more than 99.9%. In some embodiments, the transfection efficiency can be or can be about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or more than 99.9% than the transfection efficiency of comparable cells using a control delivery platform (e.g., a virus engineered absent the methods provided herein). In some cases, transfection or transduction efficiency can be quantified absent a cellular selection, sorting, or the like.

[00108] In an aspect, a vector provided herein can be delivered *in vivo* by administration to an individual subject, typically by systemic administration (*e.g.*, intravenous, intraperitoneal, intramuscular, subdermal, or intracranial infusion).

Kits

[00109] Disclosed herein can be kits comprising viral compositions. Disclosed herein can also be kits for the treatment or prevention of a cancer, pathogen infection, and/or immune disorder. In one embodiment, a kit can include a therapeutic or prophylactic composition containing an effective amount of a viral composition in unit dosage form. In some embodiments, a kit comprises a sterile container which can contain a therapeutic composition of virus; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments. In some cases, a virus provided herein, can be provided together with instructions for administering the virus to a subject having or at risk of developing a cancer, pathogen infection, immune disorder or that will undergo transplant. Instructions can generally include information about the use of the composition for the treatment or prevention of cancer, pathogen infection, immune disorder or transplant.

EXAMPLES

[00110] The examples below further illustrate the described embodiments without limiting the scope of this disclosure.

EXAMPLE 1: VECTOR DESIGN AND SEQUENCES OF A VIRAL CONSTRUCT COMPRISING A NON-REPLICATING LENTIVIRUS

[00111] A viral vector comprising a non-replicating lentivirus was generated as follows. The *env*, packaging, and transfer elements were integrated into the wild type vaccinia virus (VV) genome, vaccinia virus Western Reserve strain with GenBank accession number

AY243312.1 (**Table 1** and **SEQ ID NO: 1**), separated by tens of thousands of bases all, as shown in **FIG. 1**. The viral vector contains a hybrid 5' LTR region that is a fusion between a vaccinia virus promoter and an HIV-1 5' LTR that functions absent TAT for transcription by utilizing vaccinia virus transcription factors. Vaccinia virus strong early promoters were added for each component and a strong early promoter was fused to the 5' LTR of the transfer construct. The termination of vaccinia virus early gene transcription utilizes the termination sequence UUUUUNU (U5NU) (SEQ ID NO: 7) to attract vaccinia virus -specific factors to halt transcription and generate poly(A) tails to protect viral mRNA. The sequence ATTTTTAT (SEQ ID NO: 8) was added to the desired terminal regions of each construct.

env construct

[00112] The *vsvg*-containing *env* construct was designed to recombine with the vaccinia virus genome at a point between the *vacwr032* (*k1l*) and *vacwr033* (*k2l*) genes. A strong vaccinia virus early promoter was used:

TATTTATATTCCAAAAAAAAAAAATAAAATTTCAATTTTT (SEQ ID NO: 9)

[00113] A map and sequence with elements marked in similar colors as the map is shown below in **FIG. 2** and **Table 2**.

Table 2: Map of a lentiviral *env* construct: Vacwr032 (1-790); U5NU (906-914 and 917-925); VSVG (926-2461); vaccinia virus promoter (2470-2509); loxP (2529-2562 and 3343-3376); GFP (2606-3322); Vacwr033 (3503-4416)

SEQ ID	ID	Sequence
2	Lentiviral *env* construct	CACAATTGACGTACATGAGTCTGAGTTCCTTGTTTTTGCTAATTATTTCATCCAATTT ATTATTCTTGACTATATCGAGATCTTTTGTATAGGAGTCAGACTTGTATTCAACATGC TTTTCTATAATCATTTTAGCTATTTCGGCATCATCCAATAGTACATTTTCCAGATTAG CAGAATAGATATTAATGTCGTATTTGAACAGAGCCTGTAACATCTCAATGTCTTTATT ATCTATAGCCAATTTAATGTCCGGAATGAAGAGAAGGGAATTATTGGTGTTTGTCGAC GTCATATAGTCGAGCAAGAGAATCATCATATCCACGTGTCCATTTTTTATAGTGATGT GAATACAACTAAGGAGAATAGCCAGATCAAAAGTAGATGGTATCTCTGAAAGAAAGTA GGAAACAATACTTACATCATTAAGCATGACGGCATGATAAAATGAAGTTTTCCATCCA GTTTTCCCATAGAACATCAGTCTCCAATTTTTCTTAACAAACAGTTTTACCGTTTGCA TGTTACCACTATCAACCGCATAATACAATGCGGTGTTTCCCTTGTCATCAAATTGTGA ATCATCCAGTCCACTGAATAGCAAAATCTTTACTATTTTGGTATCTTCCAATGTGGCT GCCTGATGTAATGGAAATTCATTCTCTAGAAGATTTTTCAATGCTCCAGCGTTCAACA ACGTACATACTAGACGCACGTTATTATCAGCTATTGCATAATACAAGGCACTATGTCC ATGGACATCCGCCTTAAATGCATCTTTGCTAGAGAGGTCTGAAACGAGACGCTAATTA GTGTATATTTTTTCATTTTTTATAATTTTGTCATATTGCACCAGAATTAATAATATCT CTAATAGATCTGATTAGTAGATACATGGCTATCGCATAAAAATTGCATAAAAATTTTA CTTTCCAAGTCGGTTCATCTCTATGTCTGTATAAATCTGTCTTTTCTTGGTGTGCTTT AATTTAATGCAAAGATGGATACCAACTCGGAGAACCAAGAATAGTCCAATGATTAACC CTATGATAAAGAAGAAAGAGGCAATAGAGCTTTTCCAACTACTGAACCAACCTTCTAC AAGCTCGATTGGATTTTTGGATAGCCCAGTATCACCAAAGAATAAACTCTCATCATCA GGAAGTTGCGAAGCAGCGTCTTGAATGTGAGGATGTTCGAACACCTGAGCCTTTGAGC TAAGATGAAGATCGGAGTCCAACATACCATGTCCAATCATGTATAAAGGAAACTTATA TCCTGAACTGGTCCTCAGAACTCCATTGGGTCCAATTTCCACGTCTTCATATGGTGCC CAGTCATCCCACAGTTCCCTTTCTGTGGTAGTTCCACTGATCATTCCGACCATTCTTG

		AGAGGATTGGAGCAGCAATATCGACTCTGATGTATCTGGTCTCAAAGTATTTTAGGGT ACCATTGATTATGGTGAAAGCAGGACCGGTTCCTGGGTTTTTAGGAGCAAGATAGCTG AGATCCACTGGAGAGATTGGAAGACCCGCTCTGATTTTGCTCCAGGTTTCTTGGCAGA GGGAATAATCCAAGATCCTCTCAACGTCCTGAATTAGACTTACATCCACTGAGGTCTG AGATGGAGCAGAGATACTTGACCCTTCTGGGCATTCAGGGAATCTGGCTGCAGCAAAG AGATCCTTATCAGCCATCTCGAACCAGACACCTGATGGGAGTCTGACTCCCCAATGCT TGCAGTATTGCATTTTGCAGGCCTTGCCTCCAGTTTCATAAGCAAAGTAGTTACTTCT GAACCCTGTGCCCTCCTTTCCCAGGGATGATAGCTCTCCGTCCTCTGAGAAGAAGGTG ATGTCCATGGAAATGAGGTTAGAATCACATAGCCCTTTGACCTTATAGTCAGAATGCC AGGTTGTAGAGTTATGGACAGTGGGGCATATGTAATTGCTGCATTTTCCGTTGATGAA CTGTGAATCAACCCATTCTCCTGTGTATTCATCAACCAGCACATGGTGAGGAGTCACC TGGACAATCACTGCTTCGGCATCCGTCACAGTTGCATATCCACAACTTTGAGGAGGGA AGCCTGGATTCAGCCAAGTTCCTTGTTTCGTTTGTTCAATGCTTTCCTTGCATTGTTC TACAGATGGAGTGAAGGATCGGATGGACTGTGTTATATACTTCGGTCCATACCAGCGG AAATCACAAGTAGTGACCCATTTGGAAGCATGACACATCCAACCGTCTGCTTGAATAG CCTTGTGACTCTTGGGCATTTTGACTTGTATGGCTGTGCCTATTAAGTCATTATGCCA ATTTAAATCTGAGCTTGACGGGCAATAATGGTAATTAGAAGGAACATTTTTCCAGTTT CCTTTTTGGTTGTGTGGGAAAACTATGGTGAACTTGCAATTCACCCCAATGAATAAGA AGGCTAAGTACAAAAGGCACTTCATTAGAACGTTATTTATATTCCAAAAAAAAAAAAT AAAATTTCAATTTTTGCTAGCGGGCCCTAAGGCCATAACTTCGTATAGCATACATTAT ACGAAGTTATAATTCTGCAGATGAATTTCATTTTGTTTTTTTCTATGCTATAAATGCG TAAAGGCGAAGAGCTGTTCACTGGTGTCGTCCCTATTCTGGTGGAACTGGATGGTGAT GTCAACGGTCATAAGTTTTCCGTGCGTGGCGAGGGTGAAGGTGACGCAACTAATGGTA AACTGACGCTGAAGTTCATCTGTACTACTGGTAAACTGCCGGTACCTTGGCCGACTCT GGTAACGACGCTGACTTATGGTGTTCAGTGCTTTGCTCGTTATCCGGACCATATGAAG CAGCATGACTTCTTCAAGTCCGCCATGCCGGAAGGCTATGTGCAGGAACGCACGATTT CCTTTAAGGATGACGGCACGTACAAAACGCGTGCGGAAGTGAAATTTGAAGGCGATAC CCTGGTAAACCGCATTGAGCTGAAAGGCATTGACTTTAAAGAAGACGGCAATATCCTG GGCCATAAGCTGGAATACAATTTTAACAGCCACAATGTTTACATCACCGCCGATAAAC AAAAAAATGGCATTAAAGCGAATTTTAAAATTCGCCACAACGTGGAGGATGGCAGCGT GCAGCTGGCTGATCACTACCAGCAAAACACTCCAATCGGTGATGGTCCTGTTCTGCTG CCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTAAAGATCCGAACGAGAAAC GCGATCATATGGTTCTGCTGGAGTTCGTAACCGCAGCGGGCATCACGCATGGTATGGA TGAACTGTACAAATAATTAATTAAAAGGCCGCTCGAATAACTTCGTATAGCATACATT ATACGAAGTTATGCTAGCACGATGCTCTTAGAATAACAACATATACACATTTAATAAA AATAATATTTATTAAGAAAATTCAGATTTCACGTACCCATCAATATAAATAAAATAAT GATTCCTTACACCGTACCCATATTAAGGAGATTCCACCTTACCCATAAACAATATAAA TCCAGTAATATCATGTCTGATGATGAACACAAATGGTGTATTAAATTCCAGTTTTTCA GGAGATGATCTCGCCGTAGCTACCATAATAGTAGATGCCTCTGCTACAGTTCCTTGTT CGTCGACATCTATCTTTGCATTCTGAAACATTTTATAAATATATAATGGGTCCCTAGT CATATGTTTAAACGACGCATTATCTGGATTAAACATACTAGGAGCCATCATTTCGGCT ATCGACTTAATATCCCTCTTATTTTCGATAGAAAATTTAGGGAGTTTAAGATTGTACA CTTTATTCCCTAATTGAAACGACCAATAGTCTAATTTTGCAGCCGTAATAGAATCTGT GAAATGGGTCATATTATCACCTATTGCCAGGTACATACTAATATTAGCATCCTTATAC GGAAGGCGTACCATATCATATTCTTCGTCATCGATTGTGATTGTATTTCCTTGCAATT TAGTAACTACGTTCATCATGGGAACCGTTTTCGTACCGTACTTATTAGTAAAACTAGC ATTGCGTGTTTTAGTGATATCAAACGGATATTGCCATATACCTTTAAAATATATAGTA TTAATGATTGCCCATAGAGTATTATTGTCGAGCATATTAGAATCTACTACATTAGACA TACCGGATCTACGTTCTACTATAGAATTAATTTTATTAACCGCATCTCGTCTAAAGTT TAATCTATATAGGCCGAATCTATGATATTGTTGATAATACGACGGTTTAATGCACACA GTATTATCTACGAAACTTTGATAAGTTAGATCAGTGTACGTATATTTAGATGTTTTCA GCTTAGCTAATCCTGATATTAATTCTGTAAATGCTGGACCCAGATCTCTTTTTCTCAA ATCCATAG
3	vsvg	TTACTTTCCAAGTCGGTTCATCTCTATGTCTGTATAAATCTGTCTTTTCTTGGTGTGC TTTAATTTAATGCAAAGATGGATACCAACTCGGAGAACCAAGAATAGTCCAATGATTA ACCCTATGATAAAGAAGAAAGAGGCAATAGAGCTTTTCCAACTACTGAACCAACCTTC TACAAGCTCGATTGGATTTTTGGATAGCCCAGTATCACCAAAGAATAAACTCTCATCA TCAGGAAGTTGCGAAGCAGCGTCTTGAATGTGAGGATGTTCGAACACCTGAGCCTTTG AGCTAAGATGAAGATCGGAGTCCAACATACCATGTCCAATCATGTATAAAGGAAACTT ATATCCTGAACTGGTCCTCAGAACTCCATTGGGTCCAATTTCCACGTCTTCATATGGT GCCCAGTCATCCCACAGTTCCCTTTCTGTGGTAGTTCCACTGATCATTCCGACCATTC

		TTGAGAGGATTGGAGCAGCAATATCGACTCTGATGTATCTGGTCTCAAAGTATTTTAG GGTACCATTGATTATGGTGAAAGCAGGACCGGTTCCTGGGTTTTTAGGAGCAAGATAG CTGAGATCCACTGGAGAGATTGGAAGACCCGCTCTGATTTTGCTCCAGGTTTCTTGGC AGAGGGAATAATCCAAGATCCTCTCAACGTCCTGAATTAGACTTACATCCACTGAGGT CTGAGATGGAGCAGAGATACTTGACCCTTCTGGGCATTCAGGGAATCTGGCTGCAGCA AAGAGATCCTTATCAGCCATCTCGAACCAGACACCTGATGGGAGTCTGACTCCCCAAT GCTTGCAGTATTGCATTTTGCAGGCCTTGCCTCCAGTTTCATAAGCAAAGTAGTTACT TCTGAACCCTGTGCCCTCCTTTCCCAGGGATGATAGCTCTCCGTCCTCTGAGAAGAAG GTGATGTCCATGGAAATGAGGTTAGAATCACATAGCCCTTTGACCTTATAGTCAGAAT GCCAGGTTGTAGAGTTATGGACAGTGGGGCATATGTAATTGCTGCATTTTCCGTTGAT GAACTGTGAATCAACCCATTCTCCTGTGTATTCATCAACCAGCACATGGTGAGGAGTC ACCTGGACAATCACTGCTTCGGCATCCGTCACAGTTGCATATCCACAACTTTGAGGAG GGAAGCCTGGATTCAGCCAAGTTCCTTGTTTCGTTTGTTCAATGCTTTCCTTGCATTG TTCTACAGATGGAGTGAAGGATCGGATGGACTGTGTTATATACTTCGGTCCATACCAG CGGAAATCACAAGTAGTGACCCATTTGGAAGCATGACACATCCAACCGTCTGCTTGAA TAGCCTTGTGACTCTTGGGCATTTTGACTTGTATGGCTGTGCCTATTAAGTCATTATG CCAATTTAAATCTGAGCTTGACGGGCAATAATGGTAATTAGAAGGAACATTTTTCCAG TTTCCTTTTTGGTTGTGTGGGAAAACTATGGTGAACTTGCAATTCACCCCAATGAATA AGAAGGCTAAGTACAAAAGGCACTTCAT

Packaging construct

[00114] The packaging construct was designed to recombine with the vaccinia virus genome to replace the coding sequence for *vacwr094* (*j2r*) between the *vacwr093* (*j1r*) and *vacwr033* (*j3r*) genes. This deletion was chosen because *j2r*-deletion creates an attenuated virus that preferentially infects tumor cells. Also, this insertion positions the construct near the center of the vaccinia virus genome which is relatively distant from the other constructs. An annotated map and sequence with elements marked in similar colors as the map is shown in **FIG. 3** and **Table 3**. The packaging construct utilizes a strong vaccinia virus early promoter: AAAAATTGAAATTTTATTTTTTTTTTTTGGAATATAAATA (SEQ ID NO: 10)

Table 3: Map of the lentiviral packaging construct: vacwr093 (524-985); Vaccinia virus promoter (1000-1039); gagpol (1040-5346); U5NU (5750-5757 and 5773-5780); loxP (5869-5902 and 6699-6732); GFP (5979-6698); vacwr095 (6798-7729)

SEQ ID	ID	Sequence
4	Lentiviral packaging construct	CACAATTGACGTACATGAGTCTGAGTTCCTTGTTTTTGCTAATTATTTCATCCAATTTAT TATTCTTGACTATATCGAGATCTTTTGTATAGGAGTCAGACTTGTATTCAACATGCTTTT CTATAATCATTTTAGCTATTTCGGCATCATCCAATAGTACATTTTCCAGATTAGCAGAAT AGATATTAATGTCGTATTTGAACAGAGCCTGTAACATCTCAATGTCTTTATTATCTATAG CCAATTTAATGTCCGGAATGAAGAGAAGGGAATTATTGGTGTTTGTCGACGTCATATAGT CGAGCAAGAGAATCATCATATCCACGTGTCCATTTTTTATAGTGATGTGAATACAACTAA GGAGAATAGCCAGATCAAAAGTAGATGGTATCTCTGAAAGAAAGTAGGAAACAATACTTA CATCATTAAGCATGACGGCATGATAAAATGAAGTTTTCCATCCAGTTTTCCCATAGAACA TCAGTCTCCAATTTTTCTTAACAAACAGTTTTACCGTTTGCATGTTACCACTATCAACCG CATAATACAATGCGGTGTTTCCCTTGTCATCAAATTGTGAATCATCCAGTCCACTGAATA GCAAAATCTTTACTATTTTGGTATCTTCCAATGTGGCTGCCTGATGTAATGGAAATTCAT TCTCTAGAAGATTTTTCAATGCTCCAGCGTTCAACAACGTACATACTAGACGCACGTTAT TATCAGCTATTGCATAATACAAGGCACTATGTCCATGGACATCCGCCTTAAATGCATCTT TGCTAGAGAGGTCTGAAACGAGACGCTAATTAGTGTATATTTTTTCATTTTTTATAATTT TGTCATATTGCACCAGAATTAATAATATCTCTAATAGATCTGATTAGTAGATACATGGCT ATCGCATAAAAATTGCATAAAAATTTTACTTTCCAAGTCGGTTCATCTCTATGTCTGTAT

		AAATCTGTCTTTTCTTGGTGTGCTTTAATTTAATGCAAAGATGGATACCAACTCGGAGAA CCAAGAATAGTCCAATGATTAACCCTATGATAAAGAAGAAAGAGGCAATAGAGCTTTTCC AACTACTGAACCAACCTTCTACAAGCTCGATTGGATTTTTGGATAGCCCAGTATCACCAA AGAATAAACTCTCATCATCAGGAAGTTGCGAAGCAGCGTCTTGAATGTGAGGATGTTCGA ACACCTGAGCCTTTGAGCTAAGATGAAGATCGGAGTCCAACATACCATGTCCAATCATGT ATAAAGGAAACTTATATCCTGAACTGGTCCTCAGAACTCCATTGGGTCCAATTTCCACGT CTTCATATGGTGCCCAGTCATCCCACAGTTCCCTTTCTGTGGTAGTTCCACTGATCATTC CGACCATTCTTGAGAGGATTGGAGCAGCAATATCGACTCTGATGTATCTGGTCTCAAAGT ATTTTAGGGTACCATTGATTATGGTGAAAGCAGGACCGGTTCCTGGGTTTTTAGGAGCAA GATAGCTGAGATCCACTGGAGAGATTGGAAGACCCGCTCTGATTTTGCTCCAGGTTTCTT GGCAGAGGGAATAATCCAAGATCCTCTCAACGTCCTGAATTAGACTTACATCCACTGAGG TCTGAGATGGAGCAGAGATACTTGACCCTTCTGGGCATTCAGGGAATCTGGCTGCAGCAA AGAGATCCTTATCAGCCATCTCGAACCAGACACCTGATGGGAGTCTGACTCCCCAATGCT TGCAGTATTGCATTTTGCAGGCCTTGCCTCCAGTTTCATAAGCAAAGTAGTTACTTCTGA ACCCTGTGCCCTCCTTTCCCAGGGATGATAGCTCTCCGTCCTCTGAGAAGAAGGTGATGT CCATGGAAATGAGGTTAGAATCACATAGCCCTTTGACCTTATAGTCAGAATGCCAGGTTG TAGAGTTATGGACAGTGGGGCATATGTAATTGCTGCATTTTCCGTTGATGAACTGTGAAT CAACCCATTCTCCTGTGTATTCATCAACCAGCACATGGTGAGGAGTCACCTGGACAATCA CTGCTTCGGCATCCGTCACAGTTGCATATCCACAACTTTGAGGAGGGAAGCCTGGATTCA GCCAAGTTCCTTGTTTCGTTTGTTCAATGCTTTCCTTGCATTGTTCTACAGATGGAGTGA AGGATCGGATGGACTGTGTTATATACTTCGGTCCATACCAGCGGAAATCACAAGTAGTGA CCCATTTGGAAGCATGACACATCCAACCGTCTGCTTGAATAGCCTTGTGACTCTTGGGCA TTTTGACTTGTATGGCTGTGCCTATTAAGTCATTATGCCAATTTAAATCTGAGCTTGACG GGCAATAATGGTAATTAGAAGGAACATTTTTCCAGTTTCCTTTTTGGTTGTGTGGGAAAA CTATGGTGAACTTGCAATTCACCCCAATGAATAAGAAGGCTAAGTACAAAAGGCACTTCA TTAGAACGTTATTTATATTCCAAAAAAAAAAAAATAAAATTTCAATTTTTGCTAGCGGGCC CTAAGGCCATAACTTCGTATAGCATACATTATACGAAGTTATAATTCTGCAGATGAATTT CATTTTGTTTTTTTCTATGCTATAAATGCGTAAAGGCGAAGAGCTGTTCACTGGTGTCGT CCCTATTCTGGTGGAACTGGATGGTGATGTCAACGGTCATAAGTTTTCCGTGCGTGGCGA GGGTGAAGGTGACGCAACTAATGGTAAACTGACGCTGAAGTTCATCTGTACTACTGGTAA ACTGCCGGTACCTTGGCCGACTCTGGTAACGACGCTGACTTATGGTGTTCAGTGCTTTGC TCGTTATCCGGACCATATGAAGCAGCATGACTTCTTCAAGTCCGCCATGCCGGAAGGCTA TGTGCAGGAACGCACGATTTCCTTTAAGGATGACGGCACGTACAAAACGCGTGCGGAAGT GAAATTTGAAGGCGATACCCTGGTAAACCGCATTGAGCTGAAAGGCATTGACTTTAAAGA AGACGGCAATATCCTGGGCCATAAGCTGGAATACAATTTTAACAGCCACAATGTTTACAT CACCGCCGATAAACAAAAAAATGGCATTAAAGCGAATTTTAAAATTCGCCACAACGTGGA GGATGGCAGCGTGCAGCTGGCTGATCACTACCAGCAAAACACTCCAATCGGTGATGGTCC TGTTCTGCTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTAAAGATCCGAA CGAGAAACGCGATCATATGGTTCTGCTGGAGTTCGTAACCGCAGCGGGCATCACGCATGG TATGGATGAACTGTACAAATAATTAATTAAAAGGCCGCTCGAATAACTTCGTATAGCATA CATTATACGAAGTTATGCTAGCACGATGCTCTTAGAATAACAACATATACACATTTAATA AAAATAATATTTATTAAGAAAATTCAGATTTCACGTACCCATCAATATAAATAAAATAAT GATTCCTTACACCGTACCCATATTAAGGAGATTCCACCTTACCCATAAACAATATAAATC CAGTAATATCATGTCTGATGATGAACACAAATGGTGTATTAAATTCCAGTTTTTCAGGAG ATGATCTCGCCGTAGCTACCATAATAGTAGATGCCTCTGCTACAGTTCCTTGTTCGTCGA CATCTATCTTTGCATTCTGAAACATTTTATAAATATATAATGGGTCCCTAGTCATATGTT TAAACGACGCATTATCTGGATTAAACATACTAGGAGCCATCATTTCGGCTATCGACTTAA TATCCCTCTTATTTTCGATAGAAAATTTAGGGAGTTTAAGATTGTACACTTTATTCCCTA ATTGAAACGACCAATAGTCTAATTTTGCAGCCGTAATAGAATCTGTGAAATGGGTCATAT TATCACCTATTGCCAGGTACATACTAATATTAGCATCCTTATACGGAAGGCGTACCATAT CATATTCTTCGTCATCGATTGTGATTGTATTTCCTTGCAATTTAGTAACTACGTTCATCA TGGGAACCGTTTTCGTACCGTACTTATTAGTAAAACTAGCATTGCGTGTTTTAGTGATAT CAAACGGATATTGCCATATACCTTTAAAATATATAGTATTAATGATTGCCCATAGAGTAT TATTGTCGAGCATATTAGAATCTACTACATTAGACATACCGGATCTACGTTCTACTATAG AATTAATTTTATTAACCGCATCTCGTCTAAAGTTTAATCTATATAGGCCGAATCTATGAT ATTGTTGATAATACGACGGTTTAATGCACACAGTATTATCTACGAAACTTTGATAAGTTA GATCAGTGTACGTATATTTAGATGTTTTCAGCTTAGCTAATCCTGATATTAATTCTGTAA ATGCTGGACCCAGATCTCTTTTTCTCAAATCCATAG
5	Gagpo l	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGATGGGAAAAAATTCGG TTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAG CTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATA

		CTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAAT ACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGAAGCT TTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCT GACACAGGACACAGCAATCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAGGGG CAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAGAA GAGAAGGCTTTCAGCCCAGAAGTGATACCCATGTTTTCAGCATTATCAGAAGGAGCCACC CCACAAGATTTAAACACCATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAATG TTAAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGATAGAGTGCATCCAGTGCATGCA GGGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGACATAGCAGGAACTACT AGTACCCTTCAGGAACAAATAGGATGGATGACACATAATCCACCTATCCCAGTAGGAGAA ATCTATAAAAGATGGATAATCCTGGGATTAAATAAAATAGTAAGAATGTATAGCCCTACC AGCATTCTGGACATAAGACAAGGACCAAAGGAACCCTTTAGAGACTATGTAGACCGATTC TATAAAACTCTAAGAGCCGAGCAAGCTTCACAAGAGGTAAAAAATTGGATGACAGAAACC TTGTTGGTCCAAAATGCGAACCCAGATTGTAAGACTATTTTAAAAGCATTGGGACCAGGA GCGACACTAGAAGAAATGATGACAGCATGTCAGGGAGTGGGGGGACCCGGCCATAAAGCA AGAGTTTTGGCTGAAGCAATGAGCCAAGTAACAAATCCAGCTACCATAATGATACAGAAA GGCAATTTTAGGAACCAAAGAAAGACTGTTAAGTGTTTCAATTGTGGCAAAGAAGGGCAC ATAGCCAAAAATTGCAGGGCCCCTAGGAAAAAGGGCTGTTGGAAATGTGGAAAGGAAGGA CACCAAATGAAAGATTGTACTGAGAGACAGGCTAATTTCTTAGGGAAGATCTGGCCTTCC CACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACCAGAA GAGAGCTTCAGGTTTGGGGAAGAGACAACAACTCCCTCTCAGAAGCAGGAGCCGATAGAC AAGGAACTGTATCCTTTAGCTTCCCTCAGATCACTCTTTGGCAGCGACCCCTCGTCACAA TAAAGATAGGGGGGCAATTAAAGGAAGCTCTATTAGATACAGGAGCAGATGATACAGTAT TAGAAGAAATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGGAGGTT TTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGCGGACATAAAGCTATAG GTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAATCTGTTGACTCAGA TTGGCTGCACTTTAAATTTTCCCATTAGTCCTATTGAGACTGTACCAGTAAAATTAAAGC CAGGAATGGATGGCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCAT TAGTAGAAATTTGTACAGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAA ATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAAT TAGTAGATTTCAGAGAACTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGAA TACCACATCCTGCAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATG CATATTTCTCAGTTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTA GTATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGA AAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAAAC AAAATCCAGACATAGTCATCTATCAATACATGGATGATTTGTATGTAGGATCTGACTTAG AAATAGGGCAGCATAGAACAAAAATAGAGGAACTGAGACAACATCTGTTGAGGTGGGGAT TTACCACACCAGACAAAAAACATCAGAAAGAACCTCCATTCCTTTGGATGGGTTATGAAC TCCATCCTGATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGACAGCTGGACTG TCAATGACATACAGAAATTAGTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGA TTAAAGTAAGGCAATTATGTAAACTTCTTAGGGGAACCAAAGCACTAACAGAAGTAGTAC CACTAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCGG TACATGGAGTGTATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGC AAGGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAGT ATGCAAGAATGAAGGGTGCCCACACTAATGATGTGAAACAATTAACAGAGGCAGTACAAA AAATAGCCACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTAAATTACCCATAC AAAAGGAAACATGGGAAGCATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCCTGAGT GGGAGTTTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCCA TAATAGGAGCAGAAACTTTCTATGTAGATGGGGCAGCCAATAGGGAAACTAAATTAGGAA AAGCAGGATATGTAACTGACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGACACAACAA ATCAGAAGACTGAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTAGAAGTAA ACATAGTGACAGACTCACAATATGCATTGGGAATCATTCAAGCACAACCAGATAAGAGTG AATCAGAGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGG CATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATAAATTGGTCAGTG CTGGAATCAGGAAAGTACTATTCTTAGATGGAATAGATAAGGCCCAAGAAGAACATGAGA AATATCACAGTAATTGGAGAGCAATGGCTAGTGATTTTAACCTACCACCTGTAGTAGCAA AAGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGGGAAGCCATGCATGGACAAG TAGACTGTAGCCCAGGAATATGGCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATCT TGGTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTAATTCCAGCAGAGACAG GGCAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGAAGATGGCCAGTAAAAACAGTAC ATACAGACAATGGCAGCAATTTCACCAGTACTACAGTTAAGGCCGCCTGTTGGTGGGCGG

		GGATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAAGTCAAGGAGTAATAGAATCTA TGAATAAAGAATTAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGA CAGCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGT ACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAACTAAAGAATTAC AAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGACAGCAGAGATCCAG TTTGGAAAGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATA ATAGTGACATAAAAGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTATGGAAAAC AGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA

Transfer construct

[00115] The transfer element was inserted between *vacwr205* and *vacwr206*, a region with no late gene transcripts near the far 3' end of the vaccinia virus genome, **Table 1**. A map and sequence with elements marked in similar colors as the map is shown in **FIG. 4** and **Table 4**. The transfer construct utilizes a strong vaccinia virus early promoter:

GTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGG
GATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAA
TCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGC
GGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCT (SEQ ID NO: 11) and the human PGK promoter:

GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGCGCAGGGACGCGGC
TGCTCTGGGCGTGGTTCCGGGAAACGCAGCGGCGCCGACCCTGGGTCTCGCACA
TTCTTCACGTCCGTTCGCAGCGTCACCCGGATCTTCGCCGCTACCCTTGTGGGCCC
CCCGGCGACGCTTCCTGCTCCGCCCCTAAGTCGGGAAGGTTCCTTGCGGTTCGCG
GCGTGCCGGACGTGACAAACGGAAGCCGCACGTCTCACTAGTACCCTCGCAGAC
GGACAGCGCCAGGGAGCAATGGCAGCGCGCCGACCGCGATGGGCTGTGGCCAAT
AGCGGCTGCTCAGCAGGGCGCGCCGAGAGCAGCGGCCGGGAAGGGGCGGTGCG
GGAGGCGGGGTGTGGGGCGGTAGTGTGGGCCCTGTTCCTGCCCGCGCGGTGTTCC
GCATTCTGCAAGCCTCCGGAGCGCACGTCGGCAGTCGGCTCCCTCGTTGACCGAA
TCACCGACCTCTCTCCCCAG (SEQ ID NO: 12)

Table 4: Map of the lentiviral transfer construct: vacwr205 (1-905); 5'LTR (1012-1192); ψ (1239-1364); RRE (1857-2090); CMV promoter (2926-3129); GFP (3174-3890); cPPT/CTS (4584-4701); hPGK promoter (4750-5260); PAC (5282-5881); 3'LTR (6009-6242); U5NU (6243-6251); SV40poly(a) (6323-6331); LoxP (6469-6502 and 7332-7369); rfp (6579-7313); vacwr206 (7455-8158)

SEQ ID	ID	Sequence
6	LV transfer	CACCCGATGACAATAATGACATGGACGTAGATATTCCGTATTGTGCGACACTAGCTACCG CAAATAAAATATACGGTAGCGATAGTATCGAGTTCCACGCCTCCTTCCTACAAAAAATAA AAGACGATTTTCAAACTGTAAACTTTAATAATGCTAACCAAACAAAGGAACTAATCAACG

		AATGGGTTAAGACAATGACAAATGGTAAAATTAATTCCTTATTGACTAGTCCGCTATCCA TTAATACTCGTATGACAGTTGTTAGCGCCGTCCATTTTAAAGCAATGTGGAAATATCCAT TTTCTAAACATCTTACATATACAGACAAGTTTTATATTTCTAAGAATATAGTTACCAGTG TTGATATGATGGTGAGCACTGAGAATAACTTGCAATATGTACATATTAATGAATTATTCG GAGGATTCTCTATTATCGATATTCCATACGAGGGAAACTCTAGTATGGTAATTATACTAC CGGACGACATAGAAGGTATATATAACATAGAAAAAAATATAACAGATGAAAAATTTAAAA AATGGTGTGGTATGTTATCTACTAAAAGTATAGACTTGTATATGCCAAAGTTTAAAGTGG AAATGACAGAACCGTATAATCTGGTACCGATTTTAGAAAATTTAGGACTTACTAATATAT TCGGATATTATGCAGATTTTAGCAAGATGTGTAATGAAACTATCACTGTAGAAAAATTTC TACATACGACGTTTATAGATGTTAATGAGGAGTATACAGAAGCATCGGCCGTTACAGGAG TATTTATGACTAACTTTTCGATGGTATATCGTACGAAGGTCTACATAAACCATCCATTCA TGTACATGATTAAAGACAACACAGGACGTATACTTTTTATAGGGAAATACTGCTATCCGC AATAAATATAAACAAATAGACTTTTTATCACGTTTATCTATGTCTAAATATTACAAATAG TAATAGTATAAACTAAAGCCTAGGAAAAATTGAAACATTATATTAATCAGCGGGTCTCTC TGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAG CCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCT GGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCC CGAACAGGGACTTGAAAGCGAAAGGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGGC TTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTT TGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGA GAATTAGATCGCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAAAT TAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTGT TAGAAACATCAGAAGGCTGTAGACAAATACTGGGACAGCTACAACCATCCCTTCAGACAG GATCAGAAGAACTTAGATCATTATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAA GGATAGAGATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAA GTAAGACCACCGCACAGCAAGCGGCCGCTGATCTTCAGACCTGGAGGAGGAGATATGAGG GACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTA GCACCCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGA GCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACG CTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTG AGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTC CAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGG GGTTGCTCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGAATGCTAGTTGGAGTAAT AAATCTCTGGAACAGATTTGGAATCACACGACCTGGATGGAGTGGGACAGAGAAATTAAC AATTACACAAGCTTAATACACTCCTTAATTGAAGAATCGCAAAACCAGCAAGAAAAGAAT GAACAAGAATTATTGGAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACA AATTGGCTGTGGTATATAAAATTATTCATAATGATAGTAGGAGGCTTGGTAGGTTTAAGA ATAGTTCTTGCTGTACTTTCTATAGTGAATAGAGTTAGGCAGGGATATTCACCATTATCG TTTCAGACCCACCTCCCAACCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAA GGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCTCGACGGTATCGA TCACGAGACTAGCCTCGAGCGGCCGCCCCCTTCACCGAGGGCCTATTTCCCATGATTCCT TCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACTGTA AACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTT GCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCCAAGTACGCCCCCTATTGAC GTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTT CCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGG CAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCC ATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGT AACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATA AGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGCCACCATGCGTA AAGGCGAAGAGCTGTTCACTGGTGTCGTCCCTATTCTGGTGGAACTGGATGGTGATGTCA ACGGTCATAAGTTTTCCGTGCGTGGCGAGGGTGAAGGTGACGCAACTAATGGTAAACTGA CGCTGAAGTTCATCTGTACTACTGGTAAACTGCCGGTCCCTTGGCCGACTCTGGTAACGA CGCTGACTTATGGTGTTCAGTGCTTTGCTCGTTATCCGGACCATATGAAGCAGCATGACT TCTTCAAGTCCGCCATGCCGGAAGGCTATGTGCAGGAACGCACGATTTCCTTTAAGGATG ACGGCACGTACAAAACGCGTGCGGAAGTGAAATTTGAAGGCGATACCCTGGTAAACCGCA TTGAGCTGAAAGGCATTGACTTTAAAGAAGACGGCAATATCCTGGGCCATAAGCTGGAAT ACAATTTTAACAGCCACAATGTTTACATCACCGCCGATAAACAAAAAAATGGCATTAAAG CGAATTTTAAAATTCGCCACAACGTGGAGGATGGCAGCGTGCAGCTGGCTGATCACTACC AGCAAAACACTCCAATCGGTGATGGTCCTGTTCTGCTGCCAGACAATCACTATCTGAGCA CGCAAAGCGTTCTGTCTAAAGATCCGAACGAGAAACGCGATCATATGGTTCTGCTGGAGT

		TCGTAACCGCAGCGGGCATCACGCATGGTATGGATGAACTGTACAAATAAGAATTCGATA TCAAGCTTATCGATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTC TTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATG CTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTC TTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTG ACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCG CTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGA CAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCT TTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACG TCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGC CTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCC CGCATCGATACCGTCCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTCGACCTCGAGA CAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCA GGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAACTAAAGAATTACAAAAACAA ATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGACAGCAGAGATCCACTTTGGCCG CGGCTCGAGGGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGCGCAGGGACG CGGCTGCTCTGGGCGTGGTTCCGGGAAACGCAGCGGCGCCGACCCTGGGTCTCGCACATT CTTCACGTCCGTTCGCAGCGTCACCCGGATCTTCGCCGCTACCCTTGTGGGCCCCCCGGC GACGCTTCCTGCTCCGCCCCTAAGTCGGGAAGGTTCCTTGCGGTTCGCGGCGTGCCGGAC GTGACAAACGGAAGCCGCACGTCTCACTAGTACCCTCGCAGACGGACAGCGCCAGGGAGC AATGGCAGCGCGCCGACCGCGATGGGCTGTGGCCAATAGCGGCTGCTCAGCAGGGCGCGC CGAGAGCAGCGGCCGGGAAGGGGCGGTGCGGGAGGCGGGGTGTGGGGCGGTAGTGTGGGC CCTGTTCCTGCCCGCGCGGTGTTCCGCATTCTGCAAGCCTCCGGAGCGCACGTCGGCAGT CGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCAGGGGGATCCACCGGAGCTTAC CATGACCGAGTACAAGCCCACGGTGCGCCTCGCCACCCGCGACGACGTCCCCAGGGCCGT ACGCACCCTCGCCGCCGCGTTCGCCGACTACCCCGCCACGCGCCACACCGTCGATCCGGA CCGCCACATCGAGCGGGTCACCGAGCTGCAAGAACTCTTCCTCACGCGCGTCGGGCTCGA CATCGGCAAGGTGTGGGTCGCGGACGACGGCGCCGCGGTGGCGGTCTGGACCACGCCGGA GAGCGTCGAAGCGGGGGCGGTGTTCGCCGAGATCGGCCCGCGCATGGCCGAGTTGAGCGG TTCCCGGCTGGCCGCGCAGCAACAGATGGAAGGCCTCCTGGCGCCGCACCGGCCCAAGGA GCCCGCGTGGTTCCTGGCCACCGTCGGCGTCTCGCCCGACCACCAGGGCAAGGGTCTGGG CAGCGCCGTCGTGCTCCCCGGAGTGGAGGCGGCCGAGCGCGCCGGGGTGCCCGCCTTCCT GGAGACCTCCGCGCCCCGCAACCTCCCCTTCTACGAGCGGCTCGGCTTCACCGTCACCGC CGACGTCGAGGTGCCCGAAGGACCGCGCACCTGGTGCATGACCCGCAAGCCCGGTGCCTG ACGCCCGCCCCACGACCCGCAGCGCCCGACCGAAAGGAGCGCACGACCCCATGCATCGGT ACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTCTTAAAAGAAAA GGGGGGACTGGAAGGGCTAATTCACTCCCAACGAAGACAAGATCTGCTTTTTGCTTGTAC TGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCC ACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTT GTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAG CAAATTTTTATGTAGTAGTTCATGTCATCTTATTATTCAGTATTTATAACTTGCAAAGAA ATGAATATCAGAGAGTGAGAGGAATTTTTATAACTTGTTTATTGCAGCTTATAATGGTTA CAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAG TTGTGGTTTGTCCAAACTCATCAATGTATCTTAGGTATAGCCTCTAGAATAACTTCGTAT AGCATACATTATACGAAGTTATAATTCTGCAGATGGATCATAAAAATATAGTAGAATTTC ATTTTGTTTTTTTTCTATGCTATAAATAGCTTCTCGAGCATGGTGTCTAAGGGCGAAGAGC TGATTAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTGAACAACCACCACT TCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCA AGGTGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTCATGT ACGGCAGCAGAACCTTCATCAACCACACCCAGGGCATCCCCGATTTCTTTAAGCAGTCCT TCCCTGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGACGGGGGCGTGCTGACCG CTACCCAGGACACCAGCCTCCAGGACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGG TGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCAACA CCGAGATGCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGAACCGACATGGCCCTGAAGC TCGTGGGCGGGGGCCACCTGATCTGCAACTTCAAGACCACATACAGATCCAAGAAACCCG CTAAGAACCTCAAGATGCCCGGCGTCTACTATGTGGACCACAGACTGGAAAGAATCAAGG AGGCCGACAAAGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGACC TCCCTAGCAAACTGGGGCACAAACTTAATGGCATGGACGAGCTGTACAAGTAATTAATTA AGGCCGCTCGAATAACTTCGTATAGCATACATTATACGAAGTTATCTGATAATACTTAAA AAAATAATAATATCATTTACAATTAATAGTATAAACTAAAAATTAAACAAATCGTTATTA TAAGTAATATCAAAATGATGATATACGGATTAATAGCGTGTCTTATATTCGTGACTTCAT

		CCATCGCTAGTCCACTTTATATTCCCGTTATTCCACCCATTTCGGAAGATAAATCGTTCA ATAGTGTAGAGGTATTAGTTTCCTTGTTTAGAGATGACCAAAAAGACTATACGGTAACTT CTCAGTTCAATAACTACACTATCGATACCAAAGACTGGACTATCGGCGTACTATCCACAC CTGATGGTTTGGATATACCATTGACTAATATAACTTATTGGTCACGGTTTACTATAGGTC GTGCATTGTTCAAATCAGAGTCTGAGGATATTTTCCAAAAGAAAATGAGTATTCTAGGTG TTTCTATAGAATGTAAGAAGTCGTCGACATTACTTACTTTTTTGACCGTGCGTAAAATGA CTCGAGTATTTAATAAATTTCCAGATATGGCTTATTATCGAGGAGACTGTTTAAAAGCCG TTTATGTAACAATGACTTATAAAAATACTAAAACTGGAGAGACTGATTACACGTACCTCT CTAATGGGGGGTTGCCTGCATACTATCGTAATGGGGTCGATGGTTGATTATTGATTAGTA TATTCCTTATTCTTTTTATTCACACAAAAAGAACATTTTTATAAACATGAAACCACTGTC TAAATGTAATTATGATCTTGATTTATAGATGAAGATCAGCCTTTAGAGGATTTTAACC

EXAMPLE 2: GENERATION OF A VIRAL CONSTRUCT COMPRISING A NON-REPLICATING LENTIVIRUS

[00116] Viral constructs were created by combining short DNA fragment "gBlocks" synthesized by Integrated DNA Technologies, primer extensions, and/or by full plasmid synthesis products from GenScript. BS-C-1 cells were transfected with the completed plasmids and then infected with wildtype vaccinia virus of the Western Reserve strain obtained from ATCC. Constructs were added and selected for sequentially starting with the *env* construct, followed by packaging construct, and finally the transfer construct. In between each addition, the vaccinia virus reporters were removed by growing on Cre+ cells to remove sfGFP or TagRFP-T, and then reporter-negative virus plaques were selected.

Expression of *Gag/Pol*

[00117] Detection of *GAG* expression was performed by Western blot. BS-C-1 cells were infected with the indicated viruses at 5 multiplicity of infection (MOI). After 24 h, cells were processed for Western blot and *Gag* was visualized using the HRP-Conjugated Anti-HIV-1 *Gag* antibody from ViroLabs (Cat# HIVGAG-HRP, Lot# 0516P4). The expression of *gagpol* from Retro-Vac resulted in a fully mature and processed *Gag* protein, including p55, p41, and p24 as shown in **FIG. 5**. This indicated that the HIV protease (N-terminal of *pol*) was expressed and active because a successful -1 frameshift induced by the HIV-1 ribosomal frameshift signal near the beginning of the *pol* sequence. It is possible that host factors that assist this frameshift such as eukaryotic release factor 1 (eRF1) may be present in the cytoplasm and vaccinia virus factory. The frameshift is thought to occur in HIV-1 at 5% of total transcription events, and it is important that this ratio stay near 5% to create competent virus.

Expression of VSV-G

[00118] Successful expression of VSV-G from Retro-Vac was detected on Western blot. BS-C-1 cells were infected with the indicated viruses at 5 MOI. After 24 h, cells were processed

for Western blot and VSV-G was visualized using anti-VSV-G antibody (clone poly29039) from Bio legend (Cat# 903901, Lot# B232548) as shown in **FIG. 6**. VSV-G is predicted to be 57.4 kDa.

Reporter genes indicate successful retrovirus production

[00119] Once all lentiviral constructs were included in the same vaccinia virus lineage, the final product was grown on the common lentiviral growth cell line HEK293. After 24 h of infection, TagRFP-T reporter from the vaccinia virus was visualized within viral plaques using fluorescence microscopy as shown in **FIG. 7**. In addition, sfGFP reporter within the retroviral transfer construct was detected on cells scattered within viral plaques. This indicated that retrovirus was being created from vaccinia virus-infected cells and was transducing neighboring cells.

EXAMPLE 3: COLLECTION OF VIRAL PARTICLES

[00120] To isolate viral particles, about 12 x 10^6 293T cells are plated in 20 ml on a 15 cm2 plate 24 hours before transfection. In general, two 15cm plates are used. Cells should be well-maintained and of relatively low passage number. The following steps are done 1 plate at a time. 2 ml of 2X HBS is added dropwise to the DNA mixture while bubbling with a Pasteur pipette. Upon completion, allow the mixture to bubble for 12-15 seconds. Take plate of 293T out of the incubator (plate remains in incubator for as long as possible) and add transfection mixture dropwise all over the plate. Gently swirl plate from front to back and return immediately to incubator. 3.5 to 4 hours later, remove media, wash 2x with 10ml warm PBS, and add 20 ml warm D10 onto plate and place in incubator. 36-48 hours after transfection, harvest viral supernatant and spin at 2000 rpm, 7 min at 4°C in a 50ml tube. Filter viral SN through .45 um filter. Add 35ml of filtered supernatant to an ultracentrifuge tube. Balance tubes with additional media. Cover tubes with small piece of parafilm. (It is useful to titer some of the leftover supernatant to determine if there is loss of virus during concentration.) Spin tubes using a SW-28 rotor at 25,000 rpm, 90 min, 4°C. Decant liquid and leave tube upside down on Kimwipe for 10 min. Aspirate remaining media being careful not to touch bottom of tube. Add 15µl cold PBS (for embryo infections, or any volume you wish) and leave tube at 4°C O/N with no shaking. To resuspend, hold tube at angle and pipet fluid over pellet 20 times, being careful not to touch pellet with tip. It is expected that the pellet not be resuspended after this is complete. This pellet does not contain virus and can be discarded. Aliquot or use virus. Virus should be aliquoted, flash-frozen in liquid nitrogen and

stored at -80. There should be no change in titer with freezing concentrated virus. Avoid multiple freeze-thaws.

[00121] In another experiment cells are infected with virus generated from vectors described herein. To preferentially select production-enhanced mutants, medium from flasks infected with the virus pool is added to non-infected flasks. Clonal plaques are isolated after several rounds of serial infection.

EXAMPLE 4: CELL VIABILITY ASSAYS

[00122] In one experiment HeLa cells are grown to confluency in 6-well plates and infected with 1 MOI of Retro-Vac. After 24h, 1 mL of supernatant is removed and centrifuged at 800g, 500 ul of supernatant is then removed and used for viral plaque assay. During the serial dilution, samples are treated with anti-L1 NR-45114 antibody or VIG then incubated for 1h at 37°C. Dilutions are then added to confluent 6 well plates of BS-C-40 cells for plaque assay. After 1.5h, media was replaced with CM10 with 3% CMC. After 48h, cells are stained with crystal violet to count viral plaques to determine the titer of virus in the HeLa cell supernatant and the blocking ability of neutralizing antibodies.

[00123] In another experiment, HCT116 or MC38 cell lines are seeded in 96-well plates and allowed to grow until 90% confluent. Cells are then infected with 1 MOI of virus generated from viral vectors provided herein, for example comprising sequences provided herein. Each day, at 24h intervals, cell viability is tested with the CellTiter 96 Aqueous Non-radioactive Cell Proliferation Kit from Promega. Relative viability is calculated by removing the blank value average from all wells, calculating the average value of the uninfected control group, then calculating the relative value of each infected well as (A490 / average of uninfected wells).

EXAMPLE 5: TESTING OF ONCOLYTIC POTENTIAL OF A VIRAL VECTOR IN MICE

[00124] The aim of this study is to explore the effects of provided viral vectors in a murine mouse model of cancer, in comparison with other vectors, such as control vectors.

[00125] In one experiment, a viral vector that produces a non-replicating lentivirus is compared with a vehicle (buffered saline) control, and another modified vaccinia virus that does not generate lentiviral vector. A single dose of either one of the two viruses (1×10^7 PFU) or the saline control is administered to treat BALB/c mice implanted subcutaneously with pre-established RENCA tumors. Tumor volumes are monitored by caliper measurement.

[00126] In another experiment, a different mouse tumor model, C57/BL6 mice bearing subcutaneous B16 tumors, are used to test the effects of the Retro-Vac virus. A single

intravenous injection of either virus is given to the mice (1×10^8 PFU/mouse, n = 5 per group/time point) 96 hours after the tumor implantation, and then the mice are sacrificed 1 day or 2 days after the injection. Tumors are collected for quantification of the number of viral genomes per gram of tumor by qPCR.

[00127] In one experiment, the Retro-Vac vector is tested in mice bearing orthotopic (mammary fat pad) 4T1 tumors subcutaneously. Each mouse is treated with a single injection of the Retro-Vac vector at a dose of 1×10^8 PFU. The number of viruses in the tumors is quantified by bioluminescence imaging and measurement of the luciferase activity *in vivo*, every day after the injection for three days. The therapeutic effects of the viruses are also examined. Tumor volume is monitored along with mouse survival percentage.

[00128] In another experiment, the same viruses are tested in a different tumor model, C57/BL6 mice bearing B16 tumors subcutaneously.

[00129] In another experiment, viral replication capability for virus comprising a non-replicating lentivirus in different cancer cell lines *in vitro* is examined with plaque assays. Plaque-forming are quantified every 24 hours after addition of the viruses to the cell line. Results will be monitored to determine whether an enhanced effect *in vivo* can be detected.

[00130] In an effort to start to understand the mechanisms underlying the enhanced therapeutic effects conferred by CXCR4 expression, experiments are conducted using transgenic mice with B-cells depleted (referred to herein as JH). Viruses as described above are administered to Balb/C mice having B-Cells and JH mice without B-cells, both of which are implanted subcutaneously with pre-established 4T1 tumors. Bioluminescence imaging radiance is used to determine whether the Retro-Vac virus displays increased accumulation in the tumors. Flow cytometry experiments are also performed to determine whether there is entry of B cells into the tumors, but not other organs, like spleen or lymph nodes (LN), in BALB/c mice bearing 4T1 tumor subcutaneously.

[00131] In another experiment T cells are collected from the mice that have been implanted with 4T1 tumors subcutaneously and subsequently treated as described above, and their immune activity is examined by ELISpot assays that test their interferon-γ (IFNgamma) release in response to different immunogens. The tested T cells are recovered from spleens 14 days after the virus injection.

[00132] In one experiment, BALB/c mice bearing RENCA tumors subcutaneously are treated with a single intravenous injection (1×10^7 PFU) of Retro-Vac. Tumors are harvested 24 hours later, and the number of viral genomes per milligram of tissue quantified by qPCR.

[00133] In another experiment, BALB/c mice bearing RENCA tumors subcutaneously are treated with intravenous injections (1 x 10^7 PFU) of one of the same four modified viruses on day 1 and 4, and tumor volume is monitored as described above.

[00134] In another experiment lentivirus expression is assayed in tumor samples for 14 days after the virus injection as described above. It is expected that long term expression of the PD1 checkpoint inhibitor is modulated using this system.

EXAMPLE 6: RETRO-VAC WITH GAMMARETROVIRUS COMPONENTS

[00135] An exemplary Retro-Vac of this disclosure is designed, where the lentiviral components of the packaging and transfer constructs are replaced with those of a gamma retrovirus (*e.g.*, a moloney murine leukemia virus (MMLV)). Relative to lentiviral systems, gammaretroviruses like MMLV have selective infection and integration only with actively dividing cells. Thus, this approach can be expected to increase the safety profile and specificity of retroviral targeting by restricting infection to only actively dividing neoplastic cells.

EXAMPLE 7: RETRO-VAC WITH A T7 POLYMERASE EXPRESSION SYSTEM

[00136] In poxvirus systems, early gene transcription can produce low copy, shorter-lived, and less stable viral mRNA, while intermediate and late gene transcription can produce the most abundant, high copy, and stable viral mRNA. mRNA from early genes have clean transcription start sites (TSS) and end at a specific viral termination site. Intermediate and late genes, however, are modified to include a 5' poly(A) cap and can have unregulated and highly variable termination. Thus, to craft RNA suitable as retroviral gRNA from a lentiviral transfer construct, only the early gene transcription can usually be relied upon considering a precise TSS and termination are essential to retroviral RNA packaging. This limits transcription of the transfer construct to a low copy, low stability system. To circumvent these restrictions, a vaccinia virus is generated with an expression system based on the DNA-dependent RNA polymerase from T7 bacteriophage. This version of the retro-vac system can also express the T7 polymerase (SEQ ID No. 8 provides sequence of T7 RNA polymerase gene) which binds with high specificity to an 18 bp promoter upstream of the lentiviral transfer construct. Transcription can continue until stopping at a highly efficient termination site. This system generates much higher RNA copies relative to early-only VV transcription, and can also allow for greater control of RNA ends. Since transcription of the transfer construct can be a rate-limiting step in lentiviral production, the addition of this T7 polymerase system to retro-vac is contemplated to significantly increase lentiviral titers. An

example of such a construct is provided in **FIG. 8**. Example sequences for T7 RNA polymerase containing construct is provided as SEQ ID No. 7.

SEQ ID	ID	Sequence
7	T7RNA polymerase plasmid	CATTTATACATATTCCTGGAGATACACTGTTTAAAATTAGCGAGGTATTTATTCATAATA AGATAAATCCATCTTCCAGAATGGCATACATAGAAGAAGAAAAGAGAAATTTAGTTACTG CTATACAGATGTATACTCCTCCTGATGGATCATCCGGTATAGTCTTTGTGGCATCCGTTG CACAGTGTCACGAATATAAATCATATTTAGAAAAAAGATTACCGTATGATATGTATATTA TTCATGGTAAGGTCTTAGATATAGACGAAATATTAGAAAAAGTGTATTCATCACCTAATG TATCGATAATTATTTCTACTCCTTATTTGGAATCCAGCGTTACTATACGCAATGTTACAC ACATTTATGATATGGGTAAAGTTTTTGTCCCCGCTCCTTTTGGAGGATCGCAAGAATTTA TTTCTAAATCTATGAGAGATCAACGAAAAGGAAGAGTAGGAAGAGTTAATCCTGGTACAT ACGTCTATTTCTATGATCTGTCTTATATGAAGTCTATACAGCGAATAGATTCAGAATTTC TACATAATTATATATTGTACGCTAATAAGTTTAATCTAACACTCCCCGAAGATTTGTTTA TAATCCCTACAAATTTGGATATTCTATGGCGTACAAAGGAATATATAGACTCGTTCGATA TTAGTACAGAAACATGGAATAAATTATTATCCAATTATTATATGAAGATGATAGAGTATG CTAAACTTTATGTACTAAGTCCTATTCTCGCTGAGGAGTTGGATAACTTTGAGAGGACGG GAGAATTAACTAGTATTGTACGAGAAGCCATTTTATCTCTAAATTTACGAATTAAGATTT TAAATTTTAAACATAAAGATGATGATACGTATATACACTTTTGTAAAATATTATTCGGTG TCTATAACGGAACAAACGCTACTATATATTATCATAGACCTCTAACGGGATATATGAATA TGATTTCAGATACTATATTTGTTCCTGTAGATAATAACTAAAAGGCCGGCCGCTAGCTCA CTAATTCCAAACCCACCCGCTTTTTATAGTAAGTTTTTCACCCATAAATAATAAATACAA TAATTAATTTCTCGTAAAAGTAGAAAATATATTCTAATTTATTGCACGGTAAGGAAGTAG ATCATAAGGATCCATGAACACAATTAACATAGCCAAGAACGACTTCAGTGACATTGAGTT GGCTGCCATCCCGTTTAATACCTTAGCAGATCACTACGGAGAGCGACTTGCGAGAGAGCA ATTGGCCCTTGAGCATGAATCCTACGAAATGGGAGAAGCGCGATTCCGTAAGATGTTCGA GAGACAATTAAAAGCCGGTGAAGTAGCGGACAACGCCGCAGCAAAGCCACTTATCACAAC ACTACTTCCGAAGATGATAGCACGTATTAACGATTGGTTTGAGGAAGTAAAGGCCAAGAG AGGTAAGAGACCGACGGCTTTCCAATTCCTACAAGAGATCAAGCCTGAAGCGGTGGCGTA TATAACTATCAAAACGACCCTAGCGTGCCTAACTTCTGCCGATAACACGACGGTACAGGC TGTCGCTTCAGCGATTGGAAGAGCGATAGAAGATGAAGCACGTTTCGGACGAATCCGTGA TCTAGAAGCTAAACATTTTAAAAAGAACGTTGAGGAGCAGTTGAACAAACGAGTAGGTCA CGTGTACAAAAAGGCATTCATGCAAGTGGTAGAAGCCGACATGCTATCCAAGGGATTGCT TGGAGGTGAGGCCTGGTCGAGTTGGCACAAAGAGGACTCTATCCACGTCGGTGTTAGATG CATAGAGATGCTAATAGAGTCGACAGGTATGGTTTCCCTACATCGTCAAAATGCTGGAGT TGTAGGTCAGGATAGTGAAACGATCGAGCTAGCGCCGGAATACGCAGAGGCGATTGCGAC GCGAGCTGGTGCCCTTGCTGGAATTTCACCGATGTTCCAACCGTGCGTGGTACCGCCAAA ACCCTGGACAGGAATTACGGGTGGTGGTTATTGGGCCAACGGACGTCGACCACTTGCATT GGTACGAACACATTCGAAGAAAGCATTAATGCGTTACGAAGACGTATACATGCCGGAAGT ATACAAAGCAATTAATATCGCTCAGAATACCGCATGGAAAATCAACAAAAAGGTATTGGC AGTGGCTAACGTAATAACCAAGTGGAAACATTGTCCTGTTGAAGACATTCCTGCTATTGA ACGAGAAGAACTACCTATGAAGCCTGAGGACATAGACATGAATCCGGAAGCATTGACCGC GTGGAAACGAGCTGCGGCTGCTGTCTACCGAAAGGACAAGGCACGAAAATCCAGACGAAT ATCGCTAGAATTTATGTTGGAACAAGCCAATAAGTTTGCAAACCACAAGGCGATATGGTT TCCATACAATATGGATTGGCGAGGAAGAGTTTACGCTGTGTCTATGTTCAACCCACAGGG AAACGACATGACAAAAGGACTATTAACATTAGCAAAAGGAAAGCCTATCGGAAAAGAAGG TTACTATTGGTTGAAGATACACGGAGCTAATTGCGCAGGTGTCGATAAAGTCCCCTTCCC GGAACGTATAAAATTCATAGAGGAGAACCACGAAAACATCATGGCGTGCGCCAAATCCCC GCTAGAAAATACTTGGTGGGCGGAACAAGATTCTCCATTTTGCTTCTTAGCATTCTGCTT TGAATACGCAGGAGTCCAGCACCACGGACTAAGTTACAATTGTTCGCTTCCTCTAGCATT TGACGGTTCGTGCAGTGGAATACAACATTTCTCAGCCATGCTAAGAGATGAGGTGGGAGG TCGTGCGGTAAACCTATTGCCATCGGAGACCGTGCAGGATATATATGGTATCGTCGCCAA GAAAGTAAACGAGATTCTACAGGCGGATGCCATTAACGGAACCGACAATGAAGTTGTCAC TGTTACCGACGAGAACACAGGTGAAATTTCAGAAAAGGTGAAGCTTGGAACTAAAGCATT AGCCGGACAGTGGCTAGCGTATGGTGTCACTCGTAGTGTCACCAAACGTAGTGTCATGAC GCTTGCATATGGTTCCAAAGAATTCGGTTTTAGACAGCAGGTCTTGGAGGACACGATTCA ACCCGCCATAGATTCGGGTAAAGGATTGATGTTCACACAGCCTAATCAGGCTGCCGGTTA

		TATGGCCAAACTAATTTGGGAGTCTGTTAGTGTTACAGTCGTTGCCGCTGTCGAAGCCAT GAATTGGTTGAAAAGTGCCGCAAAACTACTTGCGGCCGAAGTGAAGGATAAGAAGACTGG TGAGATCCTACGAAAGAGATGCGCTGTGCATTGGGTCACTCCGGATGGTTTTCCGGTCTG GCAAGAATATAAAAAGCCGATACAAACGCGTTTGAACTTAATGTTTTTAGGACAATTCAG ATTACAACCCACCATTAACACGAATAAAGATTCGGAAATTGACGCACACAAGCAAGAATC AGGTATTGCACCAAACTTCGTGCACTCACAAGATGGTTCTCACTTAAGAAAAACGGTTGT CTGGGCTCATGAGAAGTATGGTATAGAGAGTTTTGCATTGATTCATGACTCCTTCGGTAC TATACCAGCCGACGCCGCCAATTTGTTCAAAGCGGTCCGAGAAACCATGGTAGACACTTA CGAATCTTGCGACGTCCTTGCGGACTTTTATGATCAATTCGCTGATCAGTTACATGAGTC GCAATTAGACAAGATGCCGGCATTACCGGCGAAGGGAAACTTGAACTTGAGAGATATTTT AGAATCTGATTTCGCCTTTGCCTAAAAGCTTATAACTTCGTATAGCATACATTATACGAA GTTATAATTCTGCAGATGGATCATAAAAATATAGTAGAATTTCATTTTGTTTTTTTCTAT GCTATAAATAGCTTCTCGAGCATGCGTAAAGGCGAAGAGCTGTTCACTGGTGTCGTCCCT ATTCTGGTGGAACTGGATGGTGATGTCAACGGTCATAAGTTTTCCGTGCGTGGCGAGGGT GAAGGTGACGCAACTAATGGTAAACTGACGCTGAAGTTCATCTGTACTACTGGTAAACTG CCGGTCCCTTGGCCGACTCTGGTAACGACGCTGACTTATGGTGTTCAGTGCTTTGCTCGT TATCCGGACCATATGAAGCAGCATGACTTCTTCAAGTCCGCCATGCCGGAAGGCTATGTG CAGGAACGCACGATTTCCTTTAAGGATGACGGCACGTACAAAACGCGTGCGGAAGTGAAA TTTGAAGGCGATACCCTGGTAAACCGCATTGAGCTGAAAGGCATTGACTTTAAAGAAGAC GGCAATATCCTGGGCCATAAGCTGGAATACAATTTTAACAGCCACAATGTTTACATCACC GCCGATAAACAAAAAAATGGCATTAAAGCGAATTTTAAAATTCGCCACAACGTGGAGGAT GGCAGCGTGCAGCTGGCTGATCACTACCAGCAAAACACTCCAATCGGTGATGGTCCTGTT CTGCTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTAAAGATCCGAACGAG AAACGCGATCATATGGTTCTGCTGGAGTTCGTAACCGCAGCGGGCATCACGCATGGTATG GATGAACTGTACAAATAATAAATAACTTCGTATAGCATACATTATACGAAGTTATATCAA ACTCTAATGACCACATCTTTTTTTAGAGATGAAAAATTTTCTACATCTCCTTTTGTAGAC ACGACTAAACATTTTGCAAAAAAAAGTTTATTAGTGTTTAGATAATCGTATACTTCATCA GTGTAGATAGTAAATGTGAACAAATAAAAGGTATTCTTACTCAATAGATTGGTAAATTCC ATAGAATATATTAATCCTTTCTTCTTGAGATCCCACATCATTTCAACCAGAGACGTTTTA TCCAATGATTTACCTCGTACTATACCACATACAAAACTAGATTTTGCAGTGACGTCGTAC CTGGTATTCCTACCAAACAAAATTTTACTTTTAGTTCTTTTAGAAAATTCTAAGGTAGAA TCTCTATTTGCCAATATGTCATCTATGGAATTACCACTAGCAAAAAATGATAGAAATATA TATTGATACATCGCAGCTGGTTTTGATCTACTATACTTTAAAAACGAATCAGATTCCATA ATTGCCTGTATATCATCAGCTGAAAAACTATGTTTTACACGTATTCCTTCGGCATTTCTT TTTAATGATATATCTTGTTTAGACAATGATAAAGTTATCATGTCCATGAGAGACGCGTCT CCGTATCGTATAAATATTTCATTAGATGTTAGACGCTTCATTAGGGGTATACTTCTATAA GGTTTCTTAATCAGTCCATCATTGGTTGCGTCAAGAACTACTATCGGATGTTGTTGGGTA TCTCTAGTGTTACACATGGCCTTACTAAAGTTTGGGTAAATAACTATGATATCTCTATTA ATTATAGATGCATATATTTCATTCGTCAAGGATATTAGTATCGACTTGCTATCGTCATTA ATACGTGTAATGTAATCATATAAATCATGCGATAGCCAAGGAAAATTCAAATAGATGTTC ATCATATAATCGTCGCTATAATTCATATTAATACGTTGACATTGACTAATTTGTAATATA GCCTCGCCACGAAGAAAGCTCTCGTATTCAGTTTCATC
8	T7RNA polymerase gene	ATGAACACAATTAACATAGCCAAGAACGACTTCAGTGACATTGAGTTGGCTGCCATCCCG TTTAATACCTTAGCAGATCACTACGGAGAGCGACTTGCGAGAGAGCAATTGGCCCTTGAG CATGAATCCTACGAAATGGGAGAAGCGCGATTCCGTAAGATGTTCGAGAGACAATTAAAA GCCGGTGAAGTAGCGGACAACGCCGCAGCAAAGCCACTTATCACAACACTACTTCCGAAG ATGATAGCACGTATTAACGATTGGTTTGAGGAAGTAAAGGCCAAGAGAGGTAAGAGACCG ACGGCTTTCCAATTCCTACAAGAGATCAAGCCTGAAGCGGTGGCGTATATAACTATCAAA ACGACCCTAGCGTGCCTAACTTCTGCCGATAACACGACGGTACAGGCTGTCGCTTCAGCG ATTGGAAGAGCGATAGAAGATGAAGCACGTTTCGGACGAATCCGTGATCTAGAAGCTAAA CATTTTAAAAAGAACGTTGAGGAGCAGTTGAACAAACGAGTAGGTCACGTGTACAAAAAG GCATTCATGCAAGTGGTAGAAGCCGACATGCTATCCAAGGGATTGCTTGGAGGTGAGGCC TGGTCGAGTTGGCACAAAGAGGACTCTATCCACGTCGGTGTTAGATGCATAGAGATGCTA ATAGAGTCGACAGGTATGGTTTCCCTACATCGTCAAAATGCTGGAGTTGTAGGTCAGGAT AGTGAAACGATCGAGCTAGCGCCGGAATACGCAGAGGCGATTGCGACGCGAGCTGGTGCC CTTGCTGGAATTTCACCGATGTTCCAACCGTGCGTGGTACCGCCAAAACCCTGGACAGGA ATTACGGGTGGTGGTTATTGGGCCAACGGACGTCGACCACTTGCATTGGTACGAACACAT TCGAAGAAAGCATTAATGCGTTACGAAGACGTATACATGCCGGAAGTATACAAAGCAATT AATATCGCTCAGAATACCGCATGGAAAATCAACAAAAAGGTATTGGCAGTGGCTAACGTA ATAACCAAGTGGAAACATTGTCCTGTTGAAGACATTCCTGCTATTGAACGAGAAGAACTA CCTATGAAGCCTGAGGACATAGACATGAATCCGGAAGCATTGACCGCGTGGAAACGAGCT

		GCGGCTGCTGTCTACCGAAAGGACAAGGCACGAAAATCCAGACGAATATCGCTAGAATTT ATGTTGGAACAAGCCAATAAGTTTGCAAACCACAAGGCGATATGGTTTCCATACAATATG GATTGGCGAGGAAGAGTTTACGCTGTGTCTATGTTCAACCCACAGGGAAACGACATGACA AAAGGACTATTAACATTAGCAAAAGGAAAGCCTATCGGAAAAGAAGGTTACTATTGGTTG AAGATACACGGAGCTAATTGCGCAGGTGTCGATAAAGTCCCCTTCCCGGAACGTATAAAA TTCATAGAGGAGAACCACGAAAACATCATGGCGTGCGCCAAATCCCCGCTAGAAAATACT TGGTGGGCGGAACAAGATTCTCCATTTTGCTTCTTAGCATTCTGCTTTGAATACGCAGGA GTCCAGCACCACGGACTAAGTTACAATTGTTCGCTTCCTCTAGCATTTGACGGTTCGTGC AGTGGAATACAACATTTCTCAGCCATGCTAAGAGATGAGGTGGGAGGTCGTGCGGTAAAC CTATTGCCATCGGAGACCGTGCAGGATATATATGGTATCGTCGCCAAGAAAGTAAACGAG ATTCTACAGGCGGATGCCATTAACGGAACCGACAATGAAGTTGTCACTGTTACCGACGAG AACACAGGTGAAATTTCAGAAAAGGTGAAGCTTGGAACTAAAGCATTAGCCGGACAGTGG CTAGCGTATGGTGTCACTCGTAGTGTCACCAAACGTAGTGTCATGACGCTTGCATATGGT TCCAAAGAATTCGGTTTTAGACAGCAGGTCTTGGAGGACACGATTCAACCCGCCATAGAT TCGGGTAAAGGATTGATGTTCACACAGCCTAATCAGGCTGCCGGTTATATGGCCAAACTA ATTTGGGAGTCTGTTAGTGTTACAGTCGTTGCCGCTGTCGAAGCCATGAATTGGTTGAAA AGTGCCGCAAAACTACTTGCGGCCGAAGTGAAGGATAAGAAGACTGGTGAGATCCTACGA AAGAGATGCGCTGTGCATTGGGTCACTCCGGATGGTTTTCCGGTCTGGCAAGAATATAAA AAGCCGATACAAACGCGTTTGAACTTAATGTTTTTAGGACAATTCAGATTACAACCCACC ATTAACACGAATAAAGATTCGGAAATTGACGCACACAAGCAAGAATCAGGTATTGCACCA AACTTCGTGCACTCACAAGATGGTTCTCACTTAAGAAAAACGGTTGTCTGGGCTCATGAG AAGTATGGTATAGAGAGTTTTGCATTGATTCATGACTCCTTCGGTACTATACCAGCCGAC GCCGCCAATTTGTTCAAAGCGGTCCGAGAAACCATGGTAGACACTTACGAATCTTGCGAC GTCCTTGCGGACTTTTATGATCAATTCGCTGATCAGTTACATGAGTCGCAATTAGACAAG ATGCCGGCATTACCGGCGAAGGGAAACTTGAACTTGAGAGATATTTTAGAATCTGATTTC GCCTTTGCCTAA

CLAIMS

1. A virus that produces from its genome a non-replicating retrovirus, wherein the virus is an oncolytic virus.

2. The virus of claim 1, wherein the non-replicating retrovirus comprises a transgene that codes for a non-retroviral protein.

3. A virus that produces from its genome a non-replicating retrovirus, wherein the virus is an oncolytic virus, wherein the non-replicating retrovirus comprises a transgene that codes for a non-retroviral protein, and wherein the non-replicating retrovirus is capable of expressing the non-retroviral protein in cells that are infected by the oncolytic virus and in cells that are not infected by the oncolytic virus, in a tumor microenvironment.

4. An oncolytic virus that produces from its genome a non-replicating retrovirus, wherein the non-replicating retrovirus comprises a transgene that codes for a non-lentiviral protein, and wherein the non-replicating retrovirus is capable of infecting and transducing both dividing and non-dividing cells in a target population of mammalian cells.

5. A virus comprising a transgene that codes for a non-retroviral protein, wherein the transgene is within an exogenous nucleic acid construct that further comprises a gene that codes for a protein of a non-replicating retrovirus, and wherein the virus is oncolytic.

6. A virus comprising a retroviral envelope construct, a retroviral packing construct, and a retroviral transfer construct, wherein at least one of the envelope construct, the packaging construct, or the transfer construct comprises a transgene that codes for a non-retroviral protein, and wherein the virus is an oncolytic virus.

7. The virus of claim 6, wherein the envelope construct, the packing construct, and the transfer construct are inserted at different locations within the genome of the oncolytic virus.

8. The virus of claim 6 or 7, wherein the envelope construct comprises a gene that codes for a retroviral envelope protein and the packaging construct comprises a gene that codes for a retroviral structural protein.

9. The virus of claim 8, wherein the gene that codes for the retroviral envelope protein is under the control of a first early viral promoter and the gene that codes for the retroviral structural protein is under the control of a second early viral promoter.

10. The virus of claim 8, wherein the gene that codes for the retroviral envelope protein is under the control of a first early viral promoter, a first early/late viral promoter or a

first late viral promoter; and the gene that codes for a retroviral structural protein is under the control of a second early viral promoter, a second early/late viral promoter, or a second late viral promoter.

11. The virus of any one of claims 6-10, wherein the envelope construct further comprises a termination sequence at its 5'-terminal region.

12. The virus of any one of claims 6-11, wherein the packaging construct further comprises a termination sequence at its 3'-terminal region.

13. The virus of any one of claims 6-12, wherein the transfer construct comprises a 3'-long terminal repeat region.

14. The virus of any one of claims 6-13, wherein the transfer construct comprises a hybrid 5'-long terminal repeat region.

15. The virus of claim 14, wherein the hybrid 5'-long terminal repeat region comprises a third early viral promoter and a HIV-1 protein 5'-long terminal repeat region.

16. The virus of claim 14, wherein the hybrid 5'-long terminal repeat region comprises a HIV-1 protein 5'-long terminal repeat region and a viral promoter, wherein the viral promoter is a third early viral promoter, a third early/late viral promoter, or a third late viral promoter.

17. The virus of any one of claims 6-16, wherein the transfer construct further comprises a gene that codes for a self-cleaving RNA ribozyme.

18. The virus of claim 17, wherein the gene that codes for the self-cleaving RNA ribozyme is positioned downstream to the 3'-long terminal repeat region of the transfer construct.

19. The virus of claim 18, wherein the self-cleaving ribozyme comprises a delta virus ribozyme or a hammerhead ribozyme.

20. The virus of any one of claims 9-19, wherein the packaging construct comprises a 5'-long terminal repeat region and wherein the second early viral promoter is fused to the 5'-long terminal repeat region.

21. The virus of any one of claims 6-20, wherein the envelope construct comprises a termination sequence at its 5'-terminal region.

22. The virus of any one of claims 6-21, wherein the packaging construct comprises a termination sequence at its 3'-terminal region.

23. The virus of any one of claims 6-22, wherein the transfer construct comprises a termination sequence at its 3'-terminal region.

24. The virus of any one of claims 11-23, wherein the termination sequence comprises a nucleotide sequence as set forth in SEQ ID No. 7.

25. The virus of any one of claims 8-24, wherein the retroviral structural protein comprises a mature gag-pol protein.

26. The virus of claim 25, wherein the mature gag-pol protein comprises p55, p41, and p24.

27. The virus of any one of claims 8-26, wherein the retroviral envelope protein comprises a VSV-G protein.

28. The virus of any one of claims 1-3 and 5-27, comprising an exogenous nucleic acid sequence that codes for a nucleic acid polymerase.

29. The virus of claim 28, wherein the nucleic acid polymerase is selected from the group consisting of: T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase, a RNA polymerase variant, and a DNA polymerase mutant.

30. The virus of any one of claims 1-3 and 5-27, comprising an exogenous nucleic acid sequence that codes for a bacteriophage polymerase.

31. The virus of claim 30, wherein the bacteriophage is selected from the group consisting of a T3 bacteriophage, a T7 bacteriophage and an SP6 bacteriophage.

32. The virus of claim 31, wherein a T3 bacteriophage polymerase is expressed with a T3 bacteriophage promoter, a T7 bacteriophage polymerase is expressed with a T7 bacteriophage promoter, and an SP6 bacteriophage polymerase is expressed with an SP6 bacteriophage promoter.

33. The oncolytic virus of claim 4, comprising an exogenous nucleic acid sequence that codes for a nucleic acid polymerase.

34. The oncolytic virus of claim 33, wherein the nucleic acid polymerase is selected from the group consisting of: T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase, a RNA polymerase variant, and a DNA polymerase mutant.

35. The oncolytic virus of claim 4, comprising an exogenous nucleic acid sequence that codes for a bacteriophage polymerase.

36. The oncolytic virus of claim 35, wherein the bacteriophage is selected from the group consisting of a T3 bacteriophage, a T7 bacteriophage and an SP6 bacteriophage.

37. The oncolytic virus of claim 36, wherein a T3 bacteriophage polymerase is expressed with a T3 bacteriophage promoter, a T7 bacteriophage polymerase is expressed with a T7 bacteriophage promoter, and an SP6 bacteriophage polymerase is expressed with an SP6 bacteriophage promoter.

38. A virus comprising: (i) a retroviral envelope construct inserted at a first location within the genome of the virus; and (ii) a retroviral packaging construct inserted at a second location within the genome of the virus, wherein the first location and the second location are not contiguous, wherein the envelope construct comprises a nucleic acid that codes for a retroviral envelope protein, wherein the packaging construct comprises a nucleic acid that codes for a retroviral structural protein, and wherein the virus is an oncolytic virus.

39. The virus of claim 38, further comprising (iii) a transfer construct inserted at a third location within the genome of the oncolytic virus.

40. The virus of claim 39, wherein the transfer construct comprises a transgene that codes for a non-retroviral protein.

41. The virus of claim 39 or 40, wherein the transfer construct is inserted near the 3' end of the genome of the oncolytic virus.

42. The virus of any one of claims 38-41, wherein the gene that codes for the retroviral envelope protein is under the control of a first early viral promoter and the gene that codes for the retroviral structural protein is under the control of a second early viral promoter.

43. The virus of any one of claims 38-41, wherein the gene that codes for the retroviral envelope protein is under the control of a first early viral promoter, a first early/late viral promoter or a first late viral promoter; and the gene that codes for a retroviral structural protein is under the control of a second early viral promoter, a second early/late viral promoter, or a second late viral promoter.

44. The virus of any one of claims 38-42, wherein the transfer construct comprises a hybrid 5'-long terminal repeat region.

45. The virus of claim 44, wherein the hybrid 5'-long terminal repeat region comprises a third early viral promoter and a HIV-1 protein 5'-long terminal repeat region.

46. The virus of claim 44, wherein the hybrid 5'-long terminal repeat region comprises a HIV-1 protein 5'-long terminal repeat region and a viral promoter, wherein the viral promoter is a third early viral promoter, a third early/late viral promoter, or a third late viral promoter.

47. The virus of any one of claims 39-46, wherein the transfer construct further comprises a gene that codes for a self-cleaving RNA ribozyme.

48. The virus of claim 47, wherein the gene that codes for the self-cleaving RNA ribozyme is positioned downstream to the 3'-long terminal repeat region of the transfer construct.

49. The virus of claim 48, wherein the self-cleaving ribozyme comprises a delta virus ribozyme or a hammerhead ribozyme.

50. The virus of any one of claims 42-49, wherein the packaging construct comprises a 5'-long terminal repeat region and wherein the second early viral promoter is fused to the 5'-long terminal repeat region.

51. The virus of any one of claims 38-50, wherein the nucleic acid that codes for the retroviral envelope protein comprises a nucleotide sequence as set forth in SEQ ID No. 1, or a nucleotide sequence that is at least about 70% to at least about 99% identical to the sequence set forth as SEQ ID No. 1.

52. The virus of any one of claims 38-51, wherein the nucleic acid that codes for the retroviral structural protein comprises a nucleotide sequence as set forth in SEQ ID No. 2, or a nucleotide sequence that is at least about 70% to at least about 99% identical to the sequence set forth as SEQ ID No. 2.

53. The virus of any one of claims 38-52, wherein the transfer construct comprises a 3'-long terminal repeat region.

54. The virus of any one of claims 38-53, wherein the envelope construct comprises a termination sequence at its 5'-terminal region.

55. The virus of any one of claims 38-54, wherein the packaging construct comprises a termination sequence at its 3'-terminal region.

56. The virus of any one of claims 39-55, wherein the transfer construct comprises a termination sequence at its 3'-terminal region.

57. The virus of any one of claims 53-56, wherein the termination sequence comprises nucleotide sequence as set forth in SEQ ID No. 5.

58. The virus of any one of claims 38-57, wherein the first location and the second location are separated by at least about 10,000 bases.

59. The virus of any one of claims 39-58, wherein the first location and the third location are separated by at least about 10,000 bases.

60. The virus of any one of claims 39-59, wherein the second location and the third location are separated by at least about 10,000 bases.

61. The virus of any one of claims 38-60, wherein the structural protein comprises a mature gag-pol protein.

62. The virus of claim 61, wherein the mature gag-pol protein comprises p55, p41, and p24.

63. The virus of any one of claims 38-62, wherein the envelope protein comprises a VSV-G protein.

64. The virus of any one of claims 1-63, wherein the oncolytic virus is tumor selective in replication.

65. The virus of claim 64, wherein the non-replicating retrovirus is produced selectively within a tumor microenvironment.

66. The virus of any one of claims 1-65, wherein the oncolytic virus in an oncolytic vaccinia virus.

67. The virus of claim 66, comprising the first, the second, and the third early viral promoters, wherein the first early viral promoter is a first vaccinia virus early promoter, the second early viral promoter is a second vaccinia virus early promoter, and the third early viral promoter is a third vaccinia virus early promoter.

68. The virus of claim 67, wherein the first vaccinia virus early promoter comprises a sequence as set forth in SEQ ID No. 9.

69. The virus of claim 67 or 68, wherein the second vaccinia virus early promoter comprises a sequence as set forth in SEQ ID No. 10.

70. The virus of any one of claims 67-69, wherein the third vaccinia virus early promoter comprises a sequence set forth as SEQ ID No. 11.

71. The virus of any one of claims 6-37 and 39-70, wherein the transfer construct comprises a human PGK promoter (SEQ ID No. 12).

72. The virus of claim any one of claims 66-71, wherein the envelope construct is inserted between genes vacwr032 and vacwr033 of the oncolytic vaccinia virus.

73. The virus of any one of claims 66-72, wherein the packaging construct is inserted between genes vacwr093 and vacwr095 of the oncolytic vaccinia virus.

74. The virus of any one of claims 6-73, wherein the genome of the oncolytic vaccinia virus comprises a deletion of the vacwr094 gene, and wherein the packaging construct is inserted into the location of the vacwr094 gene.

75. The virus of any one of claims 66-74, wherein the transfer construct is inserted between genes vacwr205 and vacwr206 of the oncolytic vaccinia virus.

76. The virus of any one of claims 1-75, wherein the non-retroviral protein comprises a therapeutic protein or a diagnostic protein.

77. The virus of claim 76, wherein the non-retroviral protein comprises the therapeutic protein, and wherein the therapeutic protein comprises an immune checkpoint modulator, an antibody or portion thereof, a Fc fusion protein, an anticoagulant, a blood

factor, a bone morphogenetic protein, an immunosuppressive agent, an immunostimulatory agent, an enzyme, a growth factor, a hormone, an interferon, an interleukin, a thrombolytic, an anti-angiogenic, a chemotherapeutic, an antibiotic, an antifungal, an antiviral, and any combination thereof.

78. The virus of any one of claims 1-77, wherein the retrovirus is an Alpharetrovirus, a Betaretrovirus, a Deltaretrovirus, an Epsilonretrovirus, a Gamma retrovirus, or a Lentivirus.

79. The virus of claim 78, wherein the retrovirus is the lentivirus.

80. The virus of claim 79, wherein the lentivirus is an HIV.

81. The virus of claim 78, wherein the retrovirus is the Gamma retrovirus.

82. The virus of claim 81, wherein the Gamma retrovirus is a Moloney murine leukemia virus.

83. The virus of any one of claims 78-80, comprising an exogenous nucleic acid sequence that codes for a nucleic acid polymerase.

84. The virus of claim 83, wherein the nucleic acid polymerase is selected from the group consisting of: T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase, a RNA polymerase variant, and a DNA polymerase mutant.

85. The virus of any one of claims 78-80, comprising an exogenous nucleic acid sequence that codes for a bacteriophage polymerase.

86. The virus of claim 85, wherein the bacteriophage is selected from the group consisting of a T3 bacteriophage, a T7 bacteriophage and an SP6 bacteriophage.

87. The virus of claim 86, wherein a T3 bacteriophage polymerase is expressed with a T3 bacteriophage promoter, a T7 bacteriophage polymerase is expressed with a T7 bacteriophage promoter, and an SP6 bacteriophage polymerase is expressed with an SP6 bacteriophage promoter.

88. A method of treating cancer, the method comprising: administering to a subject an oncolytic virus according to any one of claims 1-87, wherein the non-replicating retrovirus generated from the oncolytic virus is selectively targeted to a tumor tissue.

89. The method of claim 88, wherein the tumor tissue comprises a malignant neoplastic tissue.

90. An engineered producer virus that generates a non-replicating retrovirus from its genome, wherein the engineered producer virus comprises at least one of the following modifications:

i. mutation or deletion of at least one viral gene;

ii. insertion of at least one exogenous nucleic acid;

iii. altered tropism; or

iv. any combinations thereof.

91. The engineered producer virus of claim 90, wherein the non-replicating retrovirus comprises a transgene that codes for a non-retroviral protein.

92. The engineered producer virus of claim 90 or 91, wherein the producer virus is a vaccinia virus and the at least one viral gene is selected from the group consisting of: B5R, A52R (VACWR178), F13L, A36R, A34R, A33R, B8R, B18R, SPI-1, SPI-2, B15R, VGF, E3L, K3L, A41L, K7R, N1L, C12L, TK, and any combinations thereof.

93. The engineered producer virus of any one of claims 90-92, wherein the at least one exogenous nucleic acid codes for a protein selected from the group consisting of: CXCR4, CCR2, PH-20, HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, a metabolic modulating protein, a cytokine, a fusion protein comprising any combinations of the above, and a functional domain or fragment or variant thereof, or any combinations thereof.

94. The engineered producer virus of claim 93, wherein the at least one exogenous nucleic acid codes for the PIAS3.

95. The engineered producer virus of any one of claims 90-94, wherein the producer virus comprises a retroviral envelope construct, a retroviral packaging construct and a retroviral transfer construct, wherein the envelope construct, the packing construct, and the transfer construct are inserted at different locations within the genome of the producer virus.

96. The engineered producer virus of claim 95, wherein the envelope construct comprises a gene that codes for a retroviral envelope protein and the packaging construct comprises a gene that codes for a retroviral structural protein.

97. The engineered producer virus of claim 96, wherein the gene that codes for the retroviral envelope protein is under the control of a first early viral promoter and the gene that codes for the retroviral structural protein is under the control of a second early viral promoter.

98. The engineered producer virus of any one of claims 95-97, wherein the envelope construct further comprises a termination sequence at its 5'-terminal region.

99. The engineered producer virus of any one of claims 95-98, wherein the packaging construct further comprises a termination sequence at its 3'-terminal region.

100. The engineered producer virus of any one of claims 95-99, wherein the transfer construct comprises a termination sequence.

101. The engineered producer virus of any one of claims 95-100, wherein the transfer construct comprises a hybrid 5'-long terminal repeat region.

102. The engineered producer virus of claim 101, wherein the hybrid 5'-long terminal repeat region comprises a third early viral promoter and a HIV-1 protein 5'-long terminal repeat region.

103. The engineered producer virus of any one of claims 95-102, wherein the packaging construct comprises a 5'-long terminal repeat region and wherein the second early viral promoter is fused to the 5'-long terminal repeat region.

104. The engineered producer virus of any one of claims 95-103, wherein the envelope construct comprises a termination sequence at its 5'-terminal region.

105. The engineered producer virus of any one of claims 95-104, wherein the packaging construct comprises a termination sequence at its 3'-terminal region.

106. The engineered producer virus of any one of claims 95-105, wherein the transfer construct comprises a termination sequence.

107. The engineered producer virus of any one of claims 104-106, wherein the termination sequence comprises a nucleotide sequence as set forth in SEQ ID No. 7.

108. The engineered producer virus of any one of claims 96-107, wherein the retroviral structural protein comprises a mature gag-pol protein.

109. The engineered producer virus of claim 108, wherein the mature gag-pol protein comprises p55, p41, and p24.

110. The engineered producer virus of any one of claims 96-109, wherein the retroviral envelope protein comprises a VSV-G protein.

111. The engineered producer virus of any one of claims 95-110, wherein the producer virus in an oncolytic vaccinia virus.

112. The engineered producer virus of claim 111, wherein the envelope construct comprises a first early vaccinia promoter, the packaging construct comprises a second early vaccinia promoter, and the transfer construct comprises a third early vaccinia promoter.

113. The engineered producer virus of claim 112, wherein the first vaccinia virus early promoter comprises a sequence as set forth in SEQ ID No. 9.

114. The engineered producer virus of claim 112 or 113, wherein the second vaccinia virus early promoter comprises a sequence as set forth in SEQ ID No. 10.

115. The engineered producer virus of any one of claims 112-114, wherein the third vaccinia virus early promoter comprises a sequence set forth as SEQ ID No. 11.

116. The engineered producer virus of any one of claims 95-115, wherein the transfer construct comprises a human PGK promoter (SEQ ID No. 12).

117. The engineered producer virus of claim any one of claims 95-116, wherein the envelope construct is inserted between genes vacwr032 and vacwr033 of the oncolytic vaccinia virus.

118. The engineered producer virus of any one of claims 95-116, wherein the packaging construct is inserted between genes vacwr093 and vacwr095 of the oncolytic vaccinia virus.

119. The engineered producer virus of any one of claims 95-118, wherein the genome of the oncolytic vaccinia virus comprises a deletion of the vacwr094 gene, and wherein the packaging construct is inserted into the location of the vacwr094 gene.

120. The engineered producer virus of any one of claims 95-119, wherein the transfer construct is inserted between genes vacwr205 and vacwr206 of the oncolytic vaccinia virus.

121. The engineered producer virus of any one of claims 90-120, wherein the non-retroviral protein comprises a therapeutic protein or a diagnostic protein.

122. The engineered producer virus of claim 121, comprising the transgene that codes for the non-retroviral protein, wherein the non-retroviral protein comprises the therapeutic protein, and wherein the therapeutic protein comprises an immune checkpoint modulator, an antibody or portion thereof, a Fc fusion protein, an anticoagulant, a blood factor, a bone morphogenetic protein, an immunosuppressive agent, an immunostimulatory agent, an enzyme, a growth factor, a hormone, an interferon, an interleukin, a thrombolytic, an anti-angiogenic, a chemotherapeutic, an antibiotic, an antifungal, an antiviral, and any combination thereof.

123. The engineered producer virus of any one of claims 90-122, wherein the retrovirus is an Alpharetrovirus, a Betaretrovirus, a Deltaretrovirus, an Epsilonretrovirus, a Gamma retrovirus, or a Lentivirus.

124. The engineered producer virus of claim 123, wherein the retrovirus is a lentivirus.

125. The engineered producer virus of claim 124, wherein the lentivirus is an HIV.

126. The engineered producer virus of claim 123, wherein the retrovirus is the Gamma retrovirus.

127. The engineered producer virus of claim 126, wherein the Gamma retrovirus is a Moloney murine leukemia virus (MMLV).

128. A method of treating cancer, the method comprising: administering to a subject an engineered producer virus according to any one of claims 90-127, wherein the non-replicating retrovirus generated from the engineered producer virus is selectively targeted to a tumor tissue.

129. The method of claim 128, wherein the tumor tissue comprises a malignant neoplastic tissue.

130. A process for generating an oncolytic virus that produces a non-replicating retrovirus, the process comprising: growing a population of the oncolytic virus in mammalian cells, followed by adding and selecting for, sequentially, a retroviral envelope construct, a retroviral packaging construct, and a retroviral transfer construct, wherein at least one of the envelope construct, the packaging construct, or the transfer construct comprises a transgene that codes for a non-retroviral protein.

131. The process of claim 130, wherein the retrovirus is an Alpharetrovirus, a Betaretrovirus, a Deltaretrovirus, an Epsilonretrovirus, a Gamma retrovirus, or a Lentivirus.

132. The process of claim 131, wherein the retrovirus is the lentivirus.

133. The process of claim 132, wherein the lentivirus is an HIV.

134. The process of claim 131, wherein the retrovirus is the Gamma retrovirus.

135. The process of claim 134, wherein the Gamma retrovirus is a Moloney murine leukemia virus.

FIG. 1

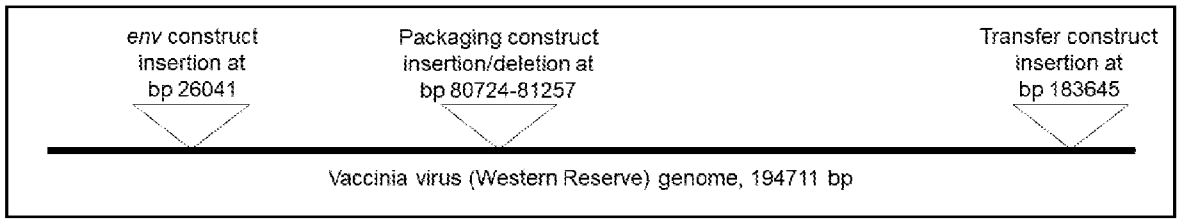

env construct insertion at bp 26041
Packaging construct insertion/deletion at bp 80724-81257
Transfer construct insertion at bp 183645
Vaccinia virus (Western Reserve) genome, 194711 bp

FIG. 2

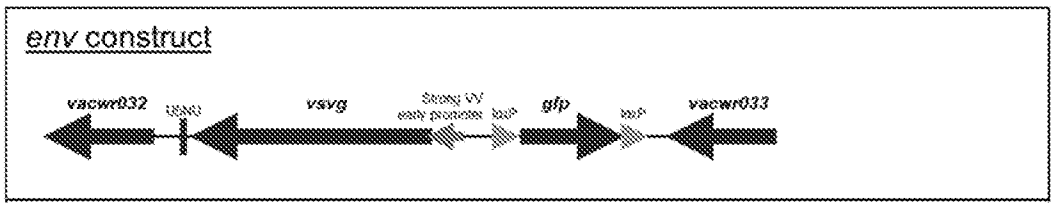


FIG. 3

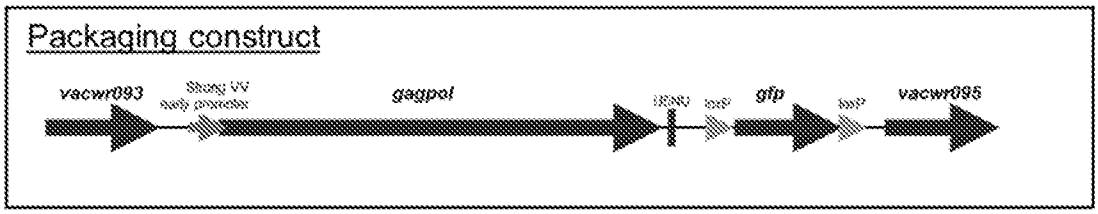


FIG. 4

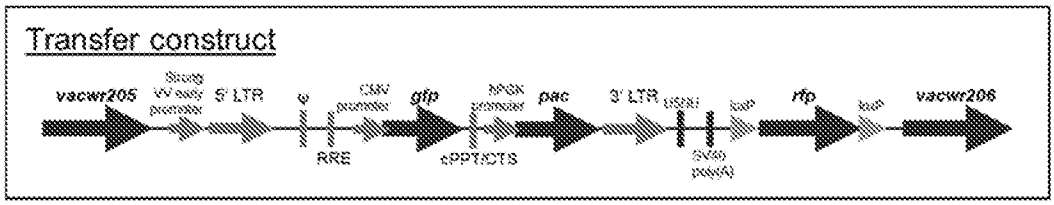

Transfer construct
vacwr205
5' LTR
ψ
RRE
gfp
cPPT/CTS
pac
3' LTR
rfp
vacwr206

FIG. 5

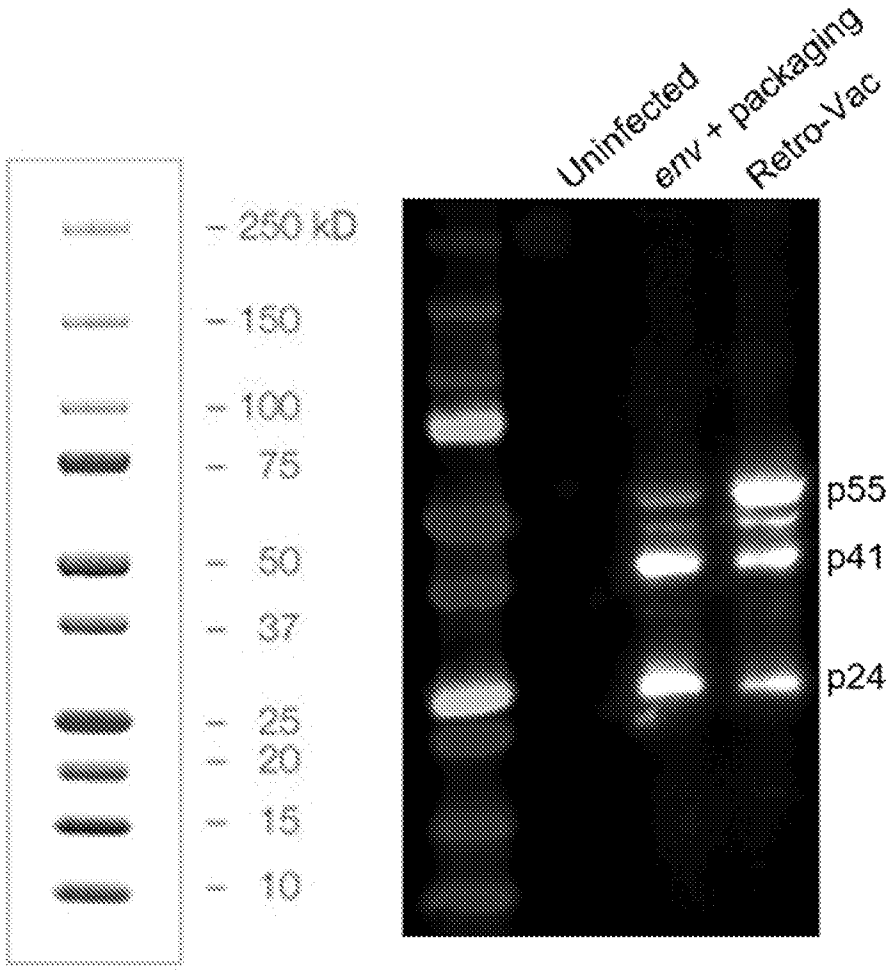

Uninfected
env + packaging
Retro-Vac
250 kD
150
100
75
50
37
25
20
15
10
p55
p41
p24

FIG. 6

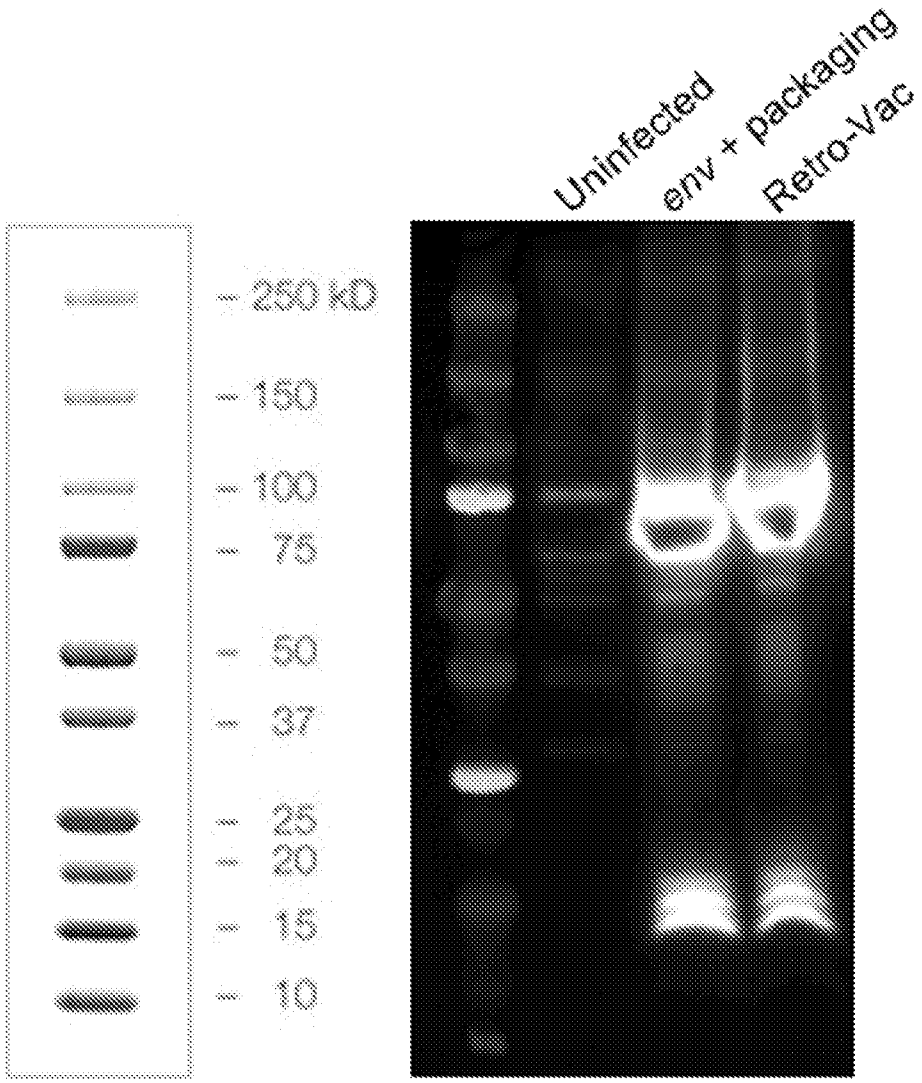

Uninfected
env + packaging
Retro-Vac
250 kD
150
100
75
50
37
25
20
15
10

FIG. 7

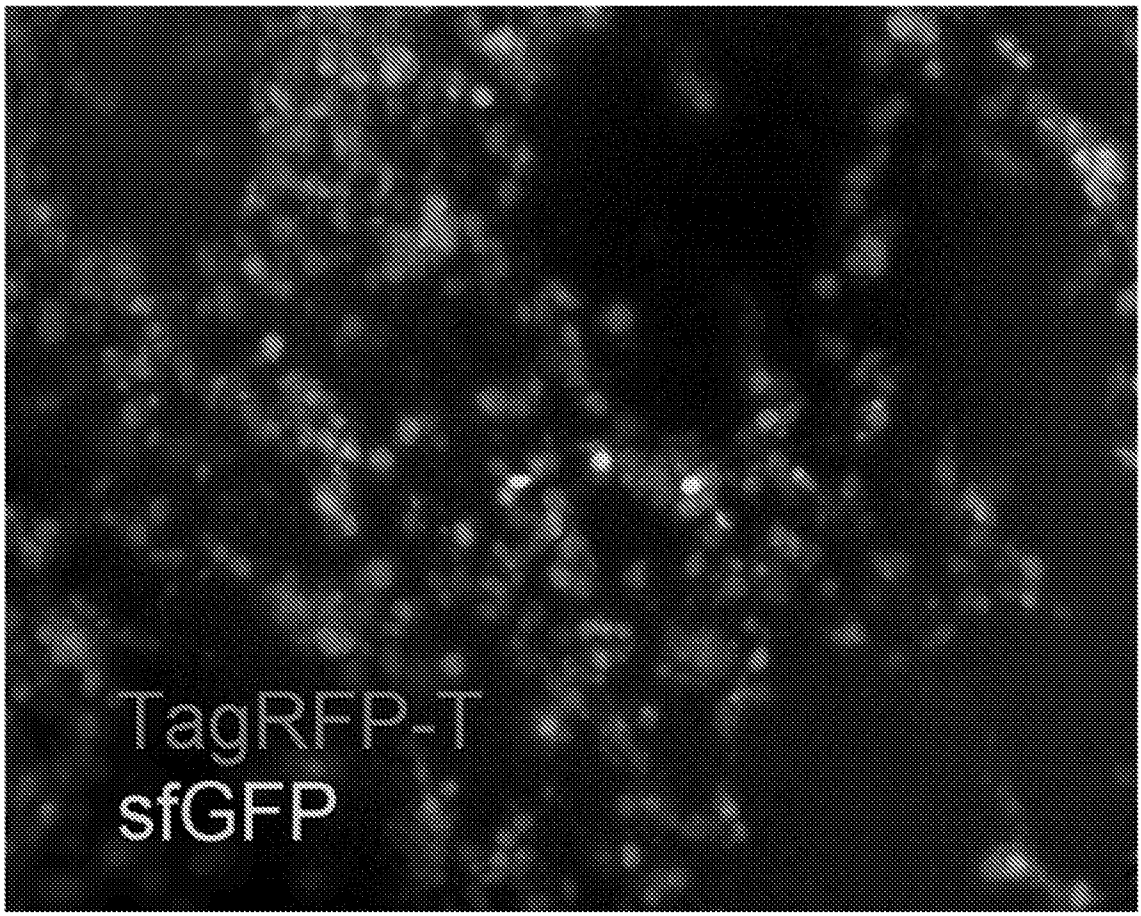
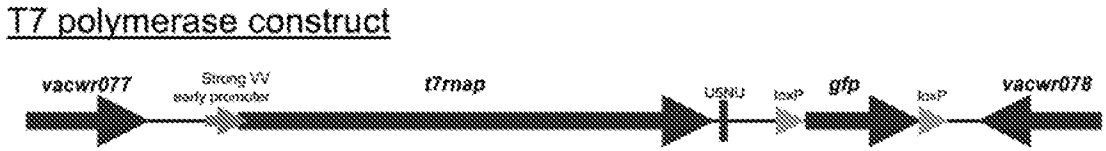


FIG. 8


T7 polymerase construct
vacwr077
Strong VV early promoter
t7rnap
USNU
loxP
gfp
loxP
vacwr078

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/056130

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 35/76; A61K 35/768; C07K 14/005; C12N 7/01; C12N 15/09; C12N 15/86 (2021.01)
CPC - C07K 14/005; C12N 2740/10043; C12N 2740/13044; C12N 2740/15043; C12N 2740/16052 (2021.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 6,352,856 B1 (FALKNER et al) 05 March 2002 (05.03.2002) entire document	1-5, 90, 91 --- 6-10, 33-41, 92, 130-135
Y	US 2007/0178592 A1 (MCARTHUR et al) 02 August 2007 (02.08.2007) entire document	6-10, 38-41, 130-135
Y	US 2010/0291139 A1 (SUTTER et al) 18 November 2010 (18.11.2010) entire document	33-37
Y	US 2019/0054131 A1 (MEMORIAL SLOAN KETTERING CANCER CENTER) 21 February 2019 (21.02.2019) entire document	92
A	US 2017/0016028 A1 (FINVECTOR VISION THERAPIES LTD.) 19 January 2017 (19.01.2017) entire document	1-10, 33-41, 90-92, 130-135

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 January 2021	08 FEB 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/056130

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 11-32, 42-89, 93-129
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.