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 mentionedin 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 ofa conflict between a term as used herein and the term as defined in the incorporated reference, the definition of this disclosure controls.

SUMMARY

[0002a] In one aspect, provided herein is a virus comprising 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, and wherein the virus is an oncolytic virus.

[0002b] In another aspect, provided herein is use of the virus as described herein in the manufacture of a medicament for the treatment of cancer.

[0002c] In another aspect, provided herein is a virus as described herein for use in therapy.

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

[0004] One examples 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 example provides a virus comprising a transgene that codes for a non-

retroviralprotein, wherein the transgene is within an exogenous nucleic acid construct that furthercomprises a gene that codes for a protein of a non-replicating retrovirus, and wherein thevirus is oncolytic. One example provides a virus comprising a retroviral envelope construct, a retroviral packing construct, and a retroviral transfer construct, wherein at leastone 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 examples, wherein the envelope construct, the packing construct, and thetransfer construct are inserted at different locations within the genome of the oncolytic virus.In some examples, 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 examples, 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 examples, the gene that codes for the retroviral envelope protein is under the control of afirst early viral promoter, a first early/late viral promoter or a first late viral promoter; and thegene 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 examples, the envelope construct further comprises a termination sequence at its 5'- terminal region. In some examples, the packaging construct further comprises a termination sequence at its 3'-terminal region. In some examples, the transfer constructcomprises a 3'-long terminal repeat region. In some examples, the transfer construct comprises a hybrid 5'-long terminal repeat region. In some examples, the hybrid 5'-longterminal repeat region comprises a third early viral promoter and a HIV-1 protein 5'-long terminal repeat region. In some examples, the hybrid 5'-long terminal repeat region comprises a HIV-1 protein 5'-long terminal repeat region and a viral promoter, wherein theviral promoter is a third early viral promoter, a third early/late viral promoter, or a third lateviral promoter. In some examples, the transfer construct further comprises a gene that codes for a self-cleaving RNA ribozyme. In some examples, the gene that codes for theself-cleaving RNA ribozyme is positioned downstream to the 3'-long terminal repeat regionof the transfer construct. In some examples, the self-cleaving ribozyme comprises a deltavirus ribozyme or a hammerhead ribozyme. In some examples, the packaging constructcomprises 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 examples, the envelope construct comprises a termination sequence at its5'-terminal region. In some examples, the packaging construct comprises a termination sequence at its 3'-terminal region. In some examples, the transfer construct comprises atermination sequence at its 3'-terminal region. In some examples, the terminationsequence comprises a nucleotide sequence as set forth in U5NU. In some examples, the retroviral structural protein comprises a mature gag-pol protein. In some examples, the mature gag-pol protein comprises p55, p41, and p24. In some

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

[0007] One example 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 packagingconstruct comprises a nucleic acid that codes for a retroviral structural protein, and whereinthe virus is an oncolytic virus. In some examples, the virus comprises (iii) a transfer construct inserted at a third location within the genome of the oncolytic virus. In some examples, the transfer construct comprises a transgene that codes for a non-retroviral protein. In some examples, the transfer construct is inserted near the 3' end of the genome of the oncolytic virus. In some examples, the gene that codes for the retroviral envelope protein is under the control of a first early viral promoter and the gene that codes forthe retroviral structural protein is under the control of a second early viral promoter. In some

examples, the gene that codes for the retroviral envelope protein is under the control of afirst early viral promoter, a first early/late viral promoter or a first late viral promoter; and thegene 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 examples, the transfer construct comprises a hybrid 5'-long terminal repeat region. In some examples, 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 examples, 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 thirdearly/late viral promoter, or a third late viral promoter. In some examples, the transfer construct further comprises a gene that codes for a self-cleaving RNA ribozyme. In some examples, 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 examples, the self-cleaving ribozyme comprises a delta virus ribozyme or a hammerheadribozyme. In some examples, 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 examples, the nucleic acid that codes for the retroviral envelope proteincomprises a nucleotide sequence as set forth in SEQ ID No. 1, or a nucleotide sequence thatis at least about 70% to at least about 99% identical to the sequence set forth as SEQ ID No.1.

[0008] In some examples, the nucleic acid that codes for the retroviral structural proteincomprises a nucleotide sequence as set forth in SEQ ID No. 2, or a nucleotide sequence thatis at least about 70% to at least about 99% identical to the sequence set forth as SEQ ID No.

2. In some examples, the transfer construct comprises a 3'-long terminal repeat region. In some examples, the envelope construct comprises a termination sequence at its 5'- terminal region. In some examples, the packaging construct comprises a termination sequence at its 3'-terminal region. In some examples, the transfer construct comprises atermination sequence at its 3'-terminal region. In some examples, the terminationsequence comprises nucleotide sequence as set forth in SEQ ID No. 5. In some examples,the first location and the second location are separated by at least about 10,000 bases. In some examples, the first location and the third location are separated by at least about 10,000 bases. In some examples, the second location and the third location are separated by at least about 10,000 bases. In some examples, the structural protein comprises a mature gag-pol protein. In some examples, the mature gag-pol protein comprises p55, p41, and

p24. In some examples, the envelope protein comprises a VSV-G protein. In some examples, the oncolytic virus is tumor selective in replication. In some examples, thenon-replicating retrovirus is produced selectively within a tumor microenvironment. In some examples, the oncolytic virus in an oncolytic vaccinia virus. In some examples, thefirst, the second, and the third early viral promoters, wherein the first early viral promoter is afirst vaccinia virus early promoter, the second early viral promoter is a second vaccinia virusearly promoter, and the third early viral promoter is a third vaccinia virus early promoter. Insome examples, the first vaccinia virus early promoter comprises a sequence as set forthin SEQ ID No. 9. In some examples, the second vaccinia virus early promoter comprisesa sequence as set forth in SEQ ID No. 10. In some examples, the third vaccinia virusearly promoter comprises a sequence set forth as SEQ ID No. 11. In some examples, thetransfer construct comprises a human PGK promoter (SEQ ID No. 12). In someexamples, the envelope construct is inserted between genes vacwr032 and vacwr033 ofthe oncolytic vaccinia virus. In some examples, the packaging construct is insertedbetween genes vacwr093 and vacwr095 of the oncolytic vaccinia virus. In someexamples, the genome of the oncolytic vaccinia virus comprises a deletion of thevacwr094 gene, and wherein the packaging construct is inserted into the location of thevacwr094 gene. In some examples, the transfer construct is inserted between genesvacwr205 and vacwr206 of the oncolytic vaccinia virus. In some examples, the non-retroviral protein comprises a therapeutic protein or a diagnostic protein. In someexamples, the non-retroviral protein comprises the therapeutic protein, and wherein thetherapeutic protein comprises an immune checkpoint modulator, an antibody or portionthereof, a Fc fusion protein, an anticoagulant, a blood factor, a bone morphogenetic protein,an immunosuppressive agent, an immunostimulatory agent, an enzyme, a growth factor, ahormone,

an interferon, an interleukin, a thrombolytic, an anti-angiogenic, a chemotherapeutic, an antibiotic, an antifungal, an antiviral, and any combination thereof. [0009] In some examples, the retrovirus is an Alpharetrovirus, a Betaretrovirus, aDeltaretrovirus, an Epsilonretrovirus, a Gamma retrovirus, or a Lentivirus. In someexamples, the retrovirus is the lentivirus. In some examples, the lentivirus is an HIV.In some examples, the retrovirus is the Gamma retrovirus. In some examples, theGamma retrovirus is a Moloney murine leukemia virus. In some examples, the viruscomprises an exogenous nucleic acid sequence that codes for a nucleic acid polymerase. Insome examples, the nucleic acid polymerase is selected from the group consisting of: T7RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase, a RNA polymerase

variant, and a DNA polymerase mutant. In some examples, the virus comprises an exogenous nucleic acid sequence that codes for a bacteriophage polymerase. In some examples, the bacteriophage is selected from the group consisting of a T3 bacteriophage,a T7 bacteriophage and an SP6 bacteriophage. In some examples, the T3 bacteriophagepolymerase 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 example a method of treating cancer, the method comprising: administeringto 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 someexamples, the tumor tissue comprises a malignant neoplastic tissue. One example 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 examples, the non-replicating lentivirus comprises a transgenethat codes for a non-retroviral protein. In some examples, the producer virus is a vacciniavirus 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 examples, the at least one exogenous nucleic acid codes for a protein selected from the group consistingof:
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 examples, the at least one exogenous nucleic acid codesfor the PIAS3.

[0011] In some examples, the producer virus comprises a retroviral envelope construct, aretroviral 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 examples, 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 examples, 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 asecond early viral promoter. In some examples, the envelope construct further comprisesa termination sequence at its 5'-terminal region.

[0012] In some examples, the packaging construct further comprises a termination sequence at its 3'-terminal region. In some examples, the transfer construct comprises atermination sequence. In some examples, the transfer construct comprises a hybrid 5'- long terminal repeat region. In some examples, the hybrid 5'-long terminal repeat regioncomprises a third early viral promoter and a HIV-1 protein 5'-long terminal repeat region. Insome examples, the packaging construct comprises a 5'-long terminal repeat region andwherein the second early viral promoter is fused to the 5'-long terminal repeat region. In some examples, the envelope construct comprises a termination sequence at its 5'- terminal region. In some examples, the packaging construct comprises a termination sequence at its 3'-terminal region. In some examples, the transfer construct comprises a termination sequence. In some examples, the termination sequence comprises a nucleotide sequence as set forth in U5NU. In some examples, the retroviral structural protein comprises a mature gag-pol protein. In some examples, the mature gag- pol protein comprises p55, p41, and p24. In some examples, the retroviral envelope protein comprises a VSV-G protein. In some examples, the producer virus in an oncolyticvaccinia virus.

[0013] In some examples, 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 examples, the firstvaccinia virus early promoter comprises a sequence as set forth in SEQ ID No. 9. In some examples, the second vaccinia virus early promoter comprises a sequence as set forth inSEQ ID No. 10. In some examples, the third vaccinia virus early promoter comprises asequence set forth as SEQ ID No. 11. In some examples, the transfer construct comprisesa human PGK promoter (SEQ ID No. 12). In some examples, the envelope construct is inserted between genes vacwr032 and vacwr033 of the oncolytic vaccinia virus. In some examples, the packaging construct is inserted between genes vacwr093 and vacwr095 ofthe oncolytic vaccinia virus. In some examples, 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 examples, the transfer construct is inserted between genes vacwr205 and vacwr206 of the oncolytic vaccinia virus. In some examples, the non-retroviral protein comprises a therapeutic protein or a diagnostic protein. In some examples, 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 orportion 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 examples, the retrovirus is an Alpharetrovirus, a Betaretrovirus, a Deltaretrovirus,an Epsilonretrovirus, a Gamma retrovirus, or a Lentivirus. In some examples, the retrovirus is a lentivirus. In some examples, the lentivirus is an HIV. In some examples, the retrovirus is the Gamma retrovirus. In some examples, the Gamma retrovirus is a Moloney murine leukemia virus (MMLV).

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

[0015] One example provides a process for generating an oncolytic virus that produces anon-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 examples, the retrovirus is an Alpharetrovirus, a Betaretrovirus, a Deltaretrovirus, an Epsilonretrovirus,a Gamma retrovirus, or a Lentivirus. In some examples, the retrovirus is the Gamma retrovirus. In some examples, the Gamma retrovirus is a Moloney murine leukemia virus.In some examples, the retrovirus is the Lentivirus. In some examples, the Lentivirusis HIV.

BRIEF DESCRIPTION OF THE DRAWINGS

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 someaspects, a third-generation vector may replace the activity of the HIV-1 protein TAT by usinga 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) 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 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 U5NU.

[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, localizedarea, 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. Forexample, 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, viralmRNA from the intermediate and late phases of infection may not be specifically terminatedor 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

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 avaccinia virus promoter and an HIV-1 5' LTR that functions absent TAT for transcription byutilizing 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) to attract vaccinia virus -specificfactors to halt transcription and generate poly(A) tails to protect viral mRNA. The sequenceATTTTTAT 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 shownbelow 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 NO:	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

		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 ofthe 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 NO:	ID	Sequence
4	Lentiviral packaging construct	CACAATTGACGTACATGAGTCTGAGTTCCTTGTTTTTGCTAATTATTTCATCCAATTTAT TATTCTTGACTATATCGAGATCTTTTGTATAGGAGTCAGACTTGTATTCAACATGCTTTT CTATAATCATTTTAGCTATTTCGGCATCATCCAATAGTACATTTTCCAGATTAGCAGAAT AGATATTAATGTCGTATTTGAACAGAGCCTGTAACATCTCAATGTCTTTATTATCTATAG CCAATTTAATGTCCGGAATGAAGAGAAGGGAATTATTGGTGTTTGTCGACGTCATATAGT CGAGCAAGAGAATCATCATATCCACGTGTCCATTTTTTATAGTGATGTGAATACAACTAA GGAGAATAGCCAGATCAAAAGTAGATGGTATCTCTGAAAGAAAGTAGGAAACAATACTTA CATCATTAAGCATGACGGCATGATAAAATGAAGTTTTCCATCCAGTTTTCCCATAGAACA TCAGTCTCCAATTTTTCTTAACAAACAGTTTTACCGTTTGCATGTTACCACTATCAACCG CATAATACAATGCGGTGTTTCCCTTGTCATCAAATTGTGAATCATCCAGTCCACTGAATA GCAAAATCTTTACTATTTTGGTATCTTCCAATGTGGCTGCCTGATGTAATGGAAATTCAT TCTCTAGAAGATTTTTCAATGCTCCAGCGTTCAACAACGTACATACTAGACGCACGTTAT TATCAGCTATTGCATAATACAAGGCACTATGTCCATGGACATCCGCCTTAAATGCATCTT TGCTAGAGAGGTCTGAAACGAGACGCTAATTAGTGTATATTTTTTCATTTTTTATAATTT TGTCATATTGCACCAGAATTAATAATATCTCTAATAGATCTGATTAGTAGATACATGGCT ATCGCATAAAAATTGCATAAAAATTTTACTTTCCAAGTCGGTTCATCTCTATGTCTGTAT

		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 NO:	ID	Sequence
6	LV transfer	CACCCGATGACAATAATGACATGGACGTAGATATTCCGTATTGTGCGACACTAGCTACCG CAAATAAAATATACGGTAGCGATAGTATCGAGTTCCACGCCTCCTTCCTACAAAAAATAA AAGACGATTTTCAAACTGTAAACTTTAATAATGCTAACCAAACAAAGGAACTAATCAACG

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

SEQ ID NO:	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