MODIFIED EXTRACELLULAR ENVELOPED VIRUS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No 62/916,035 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 individualpublication, 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 betweena 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 an oncolytic virus comprising a nucleic acid that codes for an A34R protein or a fragment thereof comprising at least two mutations that are in amino acid residues that are positively charged at pH 5, and wherein the A34R protein is mutated relative to a sequence within a wild-type A34R protein (SEQ ID No. 4).

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

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

[0003] One example provides a modified oncolytic poxvirus comprising a nucleic acid that codes for an A34R protein or a fragment thereof comprising at least two mutations, wherein the at least two mutations are in positions corresponding to positions Lys119 and Lys151 of a wild-type vaccinia virus A43R protein (SEQ ID NO. 4). In some examples, the mutation in the position corresponding to position Lys119 is Lys119Glu. In some examples, the mutation in the position corresponding to position Lys151 is Lys151Glu. In some examples, the at least two mutations in positions corresponding to positions Lys119 and Lys151 of the wild-type vaccinia virus A43R protein (SEQ ID NO. 4) are Lys119Glu and Lys151Glu, respectively.

[0004] One example provides a modified oncolytic poxvirus comprising a nucleic acid that codes for an A34R protein or a fragment thereof comprising at least two non- naturally occurring mutations that are in amino acid residues that are positively charged at pH 5, within the wild-type A34R protein (SEQ ID No. 4).

[0005] Another example provides a modified oncolytic poxvirus comprising a nucleic acid that codes for an A34R protein or a fragment thereof comprising at least two non- naturally occurring mutations that are not at position 110 of the wild-type A34R protein (SEQ ID No. 4).

[0006] Another example provides a modified oncolytic poxvirus comprising a nucleic acid that codes for an A34R protein or a fragment thereof comprising at least two non-

naturally occurring mutations that are not at aspartic acid residues within the wild-type A34R protein (SEQ ID No. 4).

[0007] Another example provides a modified oncolytic poxvirus comprising a nucleic acid that codes for an A34R protein or a fragment thereof comprising at least two non- naturally occurring mutations that are independently in alanine, arginine, asparagine, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, or valine residues within the wild-type A34R protein (SEQ ID No. 4).

[0008] Another example provides a modified oncolytic poxvirus comprising a nucleic acid that codes for an A34R protein or a fragment thereof comprising a non-naturally occurring mutation at a lysine residue that is at a position other than position Lys151 of the wild-type A34R protein (SEQ ID No. 4).

[0009] Another example provides a modified oncolytic poxvirus comprising a nucleicacid that codes for an A34R protein or a fragment thereof comprising a non-naturally occurring mutation at position Lys119 of the wild-type A34R protein (SEQ ID No. 4).

[0010] Another example provides a modified oncolytic poxvirus comprising a nucleicacid that codes for an A34R protein or a fragment thereof comprising at least two non-naturally occurring mutations that are in amino acid residues that are positively charged at pH 5, within the wild-type A34R protein (SEQ ID No. 4), wherein the modifiedoncolytic poxvirus generates an increased number of comet tail type plaques in a viralplaque forming assay, compared to an otherwise identical oncolytic virus that does not comprise the at least two non-naturally occurring mutations.

[0011] Another example provides a modified oncolytic poxvirus comprising a nucleic acid that codes for an A34R protein or a fragment thereof comprising at least two non- naturally occurring mutations, wherein if either of the non-naturally occurring mutations are at position 110 within the wild-type A34R protein (SEQ ID No. 4), the amino acid encoded is not an asparagine residue.

[0012] Another example provides a modified oncolytic poxvirus that demonstrates an increased resistance to neutralizing antibodies compared to a wild-type strain of the oncolytic poxvirus, wherein the increased resistance is measured by number of plaques produced by the modified oncolytic poxvirus or the wild-type strain in a viral plaque assay, following treatment with an anti-L1 NR-45114 antibody or an anti-VIG antibody, and wherein the modified oncolytic poxvirus produces at least about 55,000 plaque forming units/mL.

[0013] Another example provides a modified oncolytic poxvirus that produces at least about 55,000 plaque forming units/mL, in a viral plaque assay, following treatment with a neutralizing antibody.

[0014] In some examples, the neutralizing antibody is an anti-L1 NR-45114 antibody or an anti-VIG antibody. In some examples, the A34R protein or the fragment thereof further comprises a mutation at position Lys151 of the wild-type A34R protein (SEQ ID No. 4). In some examples, the amino acid residues that are positively charged at pH5 are lysine residues. In some examples, the nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to the coding sequence within the viral gene VACWR157, or a fragment thereof. In some examples, the nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to the nucleotide sequence set forth as SEQ ID No. 3. In some examples, at least one of the two non- naturally occurring mutations is at position Lys119 of the wild-type A34R protein (SEQ ID No. 4). In some examples, the non-naturally occurring mutation is at position Lys119 of the wild-type A34R protein (SEQ ID No. 4). In some examples, the mutation at position Lys119 of the wild-type A34R protein (SEQ ID No. 4) is Lys119Glu. In some examples, at least one of the two non-naturally occurring mutations is at position Lys151 of the wild-type A34R protein (SEQ ID No. 4). In some examples, the mutation at position Lys151 of the wild-type A34R protein (SEQ ID No. 4) is Lys151Glu.

[0015] One example provides a modified oncolytic poxvirus that expresses an A34R protein comprising mutations Lys119Glu and Lys151Glu

[0016] In some examples, positions 305-307 of SEQ ID No. 3 comprises nucleotides GAA or GAG. In some examples, positions 451-453 of SEQ ID No. 3 comprises nucleotides GAA or GAG. In some examples, the modified oncolytic poxvirus produces a greater amount of an extracellular enveloped virus form than an intracellular mature virus form, as compared to an otherwise identical oncolytic virus that does not comprise the at least two non-naturally occurring mutations. In some examples, the modified oncolytic poxvirus produces a greater amount of an extracellular enveloped virus form than an intracellular mature virus form, as compared to an otherwise identical oncolytic virus that does not comprise the non-naturally occurring mutation. In some examples, the modified oncolytic poxvirus further comprises an exogenous nucleic acid that codes for at least one of: a therapeutic protein or a diagnostic protein. In some examples, the exogenous nucleic acid can code for at least one of: a chemokine

receptor, a membrane associated protein, a microbial protein that is capable of degrading hyaluronan, a microbial protein, SOCS3, PH-20, HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, N1L, an immune checkpoint modulator, a metabolic modulating protein, or any combinations thereof, such as a fusion protein comprising any combination of the above (such as a metabolic modulating protein and a cytokine). In some examples, the exogenous nucleic acid that codes for a chemokine receptor, wherein the chemokine receptor comprises at least one of CXCR4 and CCR2. In some examples, the exogenous nucleic acid that codes for the membrane associated protein. In some examples, the membrane associated protein comprises a membraned associated hyaluronidase. In some examples, the membrane associated hyaluronidase comprises PH-20. In some examples, the PH-20 is GPI-anchored. In some examples, the exogenous nucleic acid that codes for the microbial protein that is capable of degrading hyaluronan, wherein the microbial protein comprises a secreted hyaluronidase. In some examples, the secreted hyaluronidase comprises at least one of HysA, lin, sko, and rv, or any combinations thereof. In some examples, the exogenous nucleic acid that codes for the microbial protein. In some examples, the microbial protein comprises HysA. In some examples, the modified oncolytic poxvirus further comprises a modification in the genome of the virus, wherein the modification comprises a mutation or a deletion of the B5R gene. In some examples, the modification in the genome of the virus, wherein the modification comprises a mutation or a deletion in a SCR region of the B5R gene, wherein said SCR region comprises SCR1, SCR3, SCR4, or any combinations thereof, and wherein the SCR region does not comprise SCR2. In some examples, the modified oncolytic poxvirus further comprises a mutation or a deletion of a viral gene selected from a group consisting of: Thymidine kinase (TK), B8R, B18R, B15R, K7R, C6L, K4L, F8L, F9L, F10L, F17R, E1L, E4L, E6R, E8R, E10R, E11L, O2L, I1L, I2L, I3L, I5L, I7L, I8R, G1L, G3L, G4L, G5.5R, G7L, G9R, L1R, L3L, L4R, L5R, J1R, J4R, J6R, H1L, H2R, H3L, H4L, H5R, H6R, D1R, D2L, D3R, D6R, D7R, D8L, D11L, D12L, D13L, A2.5L, A3L, A4L, A5R, A6L, A7L, A9L, A10L, A13L, A14L, A15L, A16L, A17L, A18R, A21L, A24R, A25L, A26L, A27L, A28L, A29L, A30L, A31R, A34R, A42R, A45R, A46R, A52R, and any combinations thereof. In some examples, the modified oncolytic poxvirus comprises the mutation or deletion of viral gene A52R. In some examples, the modifiedoncolytic poxvirus comprises (i) the exogenous nucleic acid that codes for a chemokine receptor, wherein the chemokine receptor comprises at least one of CXCR4 and CCR2;

(ii) the exogenous nucleic acid that codes for PIAS3; (iii) a mutation or deletion of the thymidine kinase gene; (iv) the mutation or deletion of the A52R gene. In some examples, the virus is suitable for systemic delivery. In some examples, the virusis capable of immune evasion. In some examples, the systemic delivery comprises oral administration, parenteral administration, intranasal administration, sublingual administration, rectal administration, transdermal administration, or any combinations thereof. In some examples, the parenteral administration comprises an intravenous injection. In some examples, the virus is suitable for intratumoral delivery. In some examples, the poxvirus is a vaccinia virus.

[0017] One example provides a process for engineering an oncolytic poxvirus comprising: (i) obtaining an oncolytic poxvirus DNA backbone vector, the oncolytic poxvirus DNA backbone vector comprising one or more modifications according to any one of the preceding claims; (ii) further modifying the oncolytic virus DNA vector to produce an engineered DNA vector; (iii) transfecting mammalian cells with the engineered DNA vector; (iv) culturing the mammalian cells under conditions suitable for viral replication; and (v) harvesting the viral particles.

[0018] In some examples, the mammalian cells comprise HeLa cells, 293 cells, A549 cells, or Vero cells.

[0019] One example provides a kit, comprising: the oncolytic poxvirus, a container; and instructions for administering said oncolytic virus to a subject to treat a disorder associated with pathological angiogenesis.

[0020] One examples provides a method of treating a tumor, the method comprising administering to a subject a therapeutically effective amount of the oncolytic poxvirus according.

[0021] One examples provides a method of treating a tumor, the method comprising administering to a subject a composition comprising patient-derived leukocyte cells infected with a modified oncolytic poxvirus that expresses an A34R protein comprising mutations at positions 119 and 151 of the wild-type A34 protein (SEQ ID No. 4), wherein the modified oncolytic poxvirus produces a population of viral particles in a tumor microenvironment. In some examples, the patient-derived leukocyte cells comprise macrophages. In some examples, the patient-derived leukocyte cells comprise tumor- targeted T cells. In some examples, at least about 10% to at least about 90% of the population of viral particles are EEV particles, as measured in a viral plaque assay. In some examples, the method further comprises harvesting the EEV particles from the

tumor microenvironment and intravenously administering the EEV particles to the subject. In some examples, the modified oncolytic poxvirus is a modified oncolytic vaccinia virus.

[0022] One example provides a process comprising infecting a culture of host cells with a population of modified oncolytic poxvirus that comprises at least about 10% to at least about 90% EEV particles, wherein the modified oncolytic poxvirus expresses an A34R protein comprising mutations at positions 119 and 151 of the wild-type A34 protein (SEQ ID No. 4). In some examples, the modified oncolytic poxvirus is a modified oncolytic vaccinia virus.

[0023] One example provides a method of treating a cancer, the method comprising administering to a patient a modified oncolytic virus comprising a nucleic acid that codesfor an A34R protein or a fragment thereof comprising at least two mutations, wherein the at least two mutations are in positions corresponding to positions Lys119 and Lys151 of a wild-type vaccinia virus A43R protein (SEQ ID NO. 4). In some examples, the at least two mutations n positions corresponding to positions Lys119 and Lys151 of a wild- type vaccinia virus A43R protein (SEQ ID NO. 4) are Lys119Glu and Lys151Glu, respectively.

[0024] One example provides a method of treating a tumor, the method comprising administering to a patient a modified oncolytic virus comprising a nucleic acid that codesfor an A34R protein or a fragment thereof comprising at least two mutations, wherein the at least two mutations are in positions corresponding to positions Lys119 and Lys151 of a wild-type vaccinia virus A43R protein (SEQ ID NO. 4). In some examples, the at least two mutations n positions corresponding to positions Lys119 and Lys151 of a wild- type vaccinia virus A43R protein (SEQ ID NO. 4) are Lys119Glu and Lys151Glu, respectively.

[0025] In some examples, the administering is via an intratumoral injection, anintravenous injection, or a combination thereof. In some examples, the administering is via an intratumoral injection, an intravenous injection, or a combination thereof.

[0026] In some examples, the method further comprises administering a further therapy, in combination with the oncolytic poxvirus, wherein the further therapycomprises at least one of: a chemotherapy, a radiation therapy, an oncolytic viral therapy with an additional virus, treatment with an immunomodulatory protein, a CAR T cellular therapy, an anti-cancer agent, an immunomodulatory agent, or any combinations thereof.

[0027] In some examples, the further therapy comprises the immunomodulatory agent selected from the group consisting of: an anti-CD33 antibody or an antigen binding fragment thereof, an anti-CD11b antibody or an antigen binding fragment thereof, a COX2 inhibitor, a cytokine, a chemokine, an anti-CTLA4 antibody or an antigen binding fragment thereof, an anti-PD-1 antibody or an antigen binding fragment thereof, an anti- PD-L1 antibody or an antigen binding fragment thereof, and a TLR agonist.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] 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.

[0029] FIG. 1 shows an exemplary assembly scheme to generate DNA for making arecombinant virus library.

[0030] FIG. 2 shows a comparison of viral plaque comet tails formed by different vacciniavirus strains.

[0031] FIGs. 3A-3B show results of neutralization assay carried out using different vacciniavirus strains (**FIG. 3A** shows results following treatment with anti-L1 NR-45114 antibody and **FIG. 3B** shows results following treatment with anti-L1R and VIG antibodies).

[0032] FIG. 4 shows results cell viability (upper panel: MC38 cells; lower panel: HCT116cells) following infection with different vaccinia virus strains.

[0033] FIG. 5 shows results of a viral replication assay in cancer cells (upper panel: HCT116cells; lower panel: MC38 cells), for different vaccinia virus strains.

DETAILED DESCRIPTION

[0034] 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 employedin practicing the disclosure. It is intended that the following claims define the scope of the

infection, and mutations in the EEV production-enhanced A34R were characterized by Sanger sequencing.

[0145] It was observed that all isolated clones shared two missense mutations: K119E and K151E, and one silent mutation at F129 (SEQ ID No. 2). The mutant protein was designated as WO34 (SEQ ID No. 2), and one corresponding clone was assigned UID WO0064R.002. The GFP reporters in this initial cohort of clones were not flanked by loxP sites and cannot beremoved. A codon-scrambled ORF encoding WO34 was synthesized and used to clone a transfer vector (pWR157-KE.R) that contained the loxP-flanked GFP. New recombinant viruses that encode WO34 were selected from fluorescent plaques and then used to infect cre recombinase-expressing cells. Reporter-lacking clones were selected after cre treatment.

Table 1: A34R gene and protein sequences

SEQ ID NO: 1	A34R	>NC_006998.1:143912-144418 Vaccinia virus, complete genome ATGAAATCGCTTAATAGACAAACTGTAAGTAGGTTTAAGAAGTTGTCGGTGC CGGCCGCTATAATGATGATACTCTCAACCATTATTAGTGGCATAGGAACATT TCTGCATTACAAAGAAGAACTGATGCCTAGTGCTTGCGCCAATGGATGGATA CAATACGATAAACATTGTTATTTAGATACTAACATTAAAATGTCTACAGATA ATGCGGTTTATCAGTGTCGTAAATTACGAGCCAGATTGCCTAGACCGGATAC TAGACATCTGAGAGTATTGTTTAGTATTTTTTATAAAGATTATTGGGTAAGT TTAAAAAAGACCAATGATAAATGGTTAGATATTAATAATGATAAAGATATAG ATATTAGTAAATTAACAAATTTTAAACAACTAAACAGTACGACGGATGCTGA AGCGTGTTATATATACAAGTCTGGAAAACTGGTTAAAACAGTATGTAAAAGT ACTCAATCTGTACTATGTGTTAAAAAATTCTACAAGTGA
SEQ ID NO: 2	A34R modified	ATGAAATCGCTTAATAGACAAACTGTAAGTAGGTTTAAGAAGTTGTCGGTGC CGGCCGCTATAATGATGATACTCTCAACCATTATTAGTGGCATAGGAACATT TCTGCATTACAAAGAAGAACTGATGCCTAGTGCTTGCGCCAATGGATGGATA CAATACGATAAACATTGTTATTTAGATACTAACATTAAAATGTCTACAGATA ATGCGGTTTATCAGTGTCGTAAATTACGAGCCAGATTGCCTAGACCGGATAC TAGACATCTGAGAGTATTGTTTAGTATTTTTTATAAAGATTATTGGGTAAGT TTAAAAAAGACCAATGATAAATGGTTAGATATTAATAATGATGAGGATATAG ATATTAGTAAATTAACAAATTTCAAACAACTAAACAGTACGACGGATGCTGA AGCGTGTTATATATACAAGTCTGGAAAACTGGTTGAAACAGTATGTAAAAGT ACTCAATCTGTACTATGTGTTAAAAAATTCTACAAGTGA
SEQ ID NO: 3	A34R mutated (nucleic acid sequences) (nucleic acid differences with wild type A34R coding sequence are bold, and mutated codons underlined	atgaaatcgcttaatagacaaactgtaagtaggtttaagaagttgtcggtgc cggccgctataatgatgatactctcaaccattattagtggcataggaacatt tctgcattacaaagaagaactgatgcctagtgcttgcgccaatggatggata caatacgataaacattgttatttagatactaacattaaaatgtctacagata atgcggtttatcagtgtcgtaaattacgagccagattgcctagaccggatac tagacatctgagagtattgtttagtatttttttataaagattattgggtaagt ttaaaaaagaccaatgataaatggttagatattaataatgat**GaG**gatatag atattagtaaattaacaaattt**C**aaacaactaaacagtacgacggatgctga agcgtgttatatatacaagtctggaaaactggtt**Gaa**acagtatgtaaaagt actcaatctgtactatgtgttaaaaaattctacaagtga

SEQ ID NO: 4	Wild-typeA34R protein	>sp|P24761|A34_VACCW Protein A34 OS=Vaccinia virus (strain Western Reserve) OX=10254 GN=VACWR157 PE=1 SV=1 MKSLNRQTVSRFKKLSVPAAIMMILSTIISGIGTFLHYKEELMPSACANGWI QYDKHCYLDTNIKMSTDNAVYQCRKLRARLPRPDTRHLRVLFSIFYKDYWVS LKKTNDKWLDINNDKDIDISKLTNFKQLNSTTDAEACYIYKSGKLVKTVCKS TQSVLCVKKFYK
SEQ ID NO: 5	WO34 (A34 double mutant K119E K151E)	MKSLNRQTVSRFKKLSVPAAIMMILSTIISGIGTFLHYKEELMPSACANGWI QYDKHCYLDTNIKMSTDNAVYQCRKLRARLPRPDTRHLRVLFSIFYKDYWVS LKKTNDKWLDINNDEDIDISKLTNFKQLNSTTDAEACYIYKSGKLVETVCKS TQSVLCVKKFYK*

Viral plaque comet tails formed by EEV-enhanced vaccinia virus containing WO34

[0146] EEV-enhanced vaccinia viruses that were previously titered were diluted and placedover confluent BS-C-40 cells in 6-well plates. Plates were incubated and left undisturbed for 48 hours and then the cells were stained with crystal violet to view differences in comet tail formation caused by aberrant EEV production. Results are shown in **FIG. 2**. As is seen in **FIG. 2**, the WO34 containing vaccinia virus strain demonstrated enhanced comet-tail formation compared to the WI strain (A34R single mutation in WR strain) and WR strain (non-mutated A34R). The comet-tail formation of the WO34 containing vaccinia virus strainwas visibly increased.

Neutralization of the EEV-enhanced vaccinia virus containing WO34

[0147] In two separate experiments using different strains of vaccinia viruses (WO34 containing vaccinia virus, WI strain, IHD-J strain, and WR strain), HeLa cells were grown to confluency in 6-well plates and infected with 1 MOI (multiplicity of infection) of a vaccinia virus strain. After 24 hours, 1 mL of culture medium. was removed and centrifuged at 800g. About 500 µl of supernatant was then removed and used for viral plaque assay. During the serial dilution, samples were treated with anti-L1 NR-45114 antibody or VIG then incubated for 1 hour at 37 °C. Dilutions were then added to confluent 6 well plates of BS-C-40 cells for plaque assay. After 1.5 hours, media was replaced with CM10 with 3% CMC. After 48 hours,cells were 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 as shown in **FIGS.3A-3B** (FIG. 3A shows viral plaque titer followed by treatment with the anti-L1 NR-45114 antibody and FIG. 3B shows the same followed by treatment with the anti-L1R antibody and the VIG antibody).

Cell viability after infection with EEV-enhanced vaccinia virus containing WO34

[0148] In 96-well plates, HCT116 or MC38 cell lines were seeded and allowed to grow until90% confluent. Cells were then infected with 1 MOI of different vaccinia virus strains (WR

CLAIMS

1. An oncolytic virus comprising a nucleic acid that codes for an A34R protein or a fragment thereof comprising at least two mutations that are in amino acid residues that are positively charged at pH 5, and wherein the A34R protein is mutated relative to a sequence within a wild-type A34R protein (SEQ ID No. 4).

2. The oncolytic virus of claim 1, wherein the at least two mutations are in positions corresponding to positions Lys119 and Lys151 of a wild-type vaccinia virus A34R protein (SEQ ID NO. 4).

3. The oncolytic virus of claim 2, wherein the mutation in the position corresponding to position Lys119 is Lys119Glu.

4. The oncolytic virus of claim 2 or 3, wherein the mutation in the position corresponding to position Lys151 is Lys151Glu.

5. The oncolytic virus of claim 2, wherein the at least two mutations in positions corresponding to positions Lys119 and Lys151 of the wild-type vaccinia virusA34R protein (SEQ ID NO. 4) are Lys119Glu and Lys151Glu, respectively.

6. The oncolytic virus of any one of claims 1-5, wherein the at least two mutations that are in amino acids residues that are positively charged at pH 5 are for amino acid residues that are negatively charged at pH 5.

7. The oncolytic virus of claim 1, wherein one of the at least two mutations is at position Lys119 of the wild-type A34R protein (SEQ ID No. 4).

8. The oncolytic virus of any one of claims 1-7, wherein the oncolytic virus produces a greater amount of an extracellular enveloped virus form than an intracellular mature virus form, as compared to an otherwise identical oncolytic virus that does not comprise the at least two mutations.

9. The oncolytic virus of any of one of claims 1-8, further comprising an exogenous nucleic acid that codes for at least one of: a therapeutic protein or a diagnostic protein.

10. The oncolytic virus of claim 9, wherein the exogenous nucleic acid cancode for at least one of: a chemokine receptor, a membrane associated protein, a microbial protein that is capable of degrading hyaluronan, a microbial protein, SOCS3, PH-20, HMGB1, PIAS3, IL15, IL15-Rα, LIGHT, ITAC, fractalkine, CCL5, N1L, an immune checkpoint modulator, a metabolic modulating protein, or any combinations thereof.

11. The oncolytic virus of claim 10, comprising the exogenous nucleic acidthat codes for a chemokine receptor, wherein the chemokine receptor comprises at least one of CXCR4 and CCR2.

12. The oncolytic virus of any one of claims 1-11, further comprising the mutation or deletion of viral gene A52R.

13. The oncolytic virus of any one of claims 1-12, wherein the oncolytic virus is modified at a genome level.

14. The oncolytic virus of any one of claims 1-13, wherein the oncolytic virus is a pox virus.

15. The oncolytic virus of claim 14, wherein the poxvirus is a vaccinia virus or a myxoma virus.

16. Use of the oncolytic virus of any one of claims 1-15, in the manufacture of a medicament for the treatment of a cancer.

17. The use of claim 16, wherein a therapeutically effective amount of an oncolytic virus according to any one of claims 1-15 is to be administered to a subject.

18. The use of claim 16 or 17, wherein the oncolytic virus is to be administered via anintratumoral injection, an intravenous injection, or a combination thereof.

19. The use of any one of claims 16-18, wherein said medicament is to be administered in combination with a further therapy, wherein the further therapy comprises at least one of: a chemotherapy, a radiation therapy, an oncolytic viral therapy withan additional virus, treatment with an immunomodulatory protein, a CAR

T cellular therapy, an anti-cancer agent, an immunomodulatory agent, or any combinations thereof.

20. The use of claim 19, wherein the further therapy comprises the immunomodulatory agent selected from the group consisting of: an anti-CD33 antibody or anantigen binding fragment thereof, an anti-CD11b antibody or an antigen binding fragment thereof, a COX2 inhibitor, a cytokine, a chemokine, an anti-CTLA4 antibody or an antigen binding fragment thereof, an anti-PD-1 antibody or an antigen binding fragment thereof, an anti-PD-L1 antibody or an antigen binding fragment thereof, and a TLR agonist.

21. An oncolytic virus of any one of claims 1-15 for use in therapy.