

CLAIMS

1. 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).
2. The modified oncolytic poxvirus of claim 1, wherein the mutation in the position corresponding to position Lys119 is Lys119Glu.
3. The modified oncolytic virus of claim 1 or 2, wherein the mutation in the position corresponding to position Lys151 is Lys151Glu.
4. The modified oncolytic virus of claim 1, wherein 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.
5. 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).
6. 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).
7. 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).
8. 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).
9. 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).

10. 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 position Lys119 of the wild-type A34R protein (SEQ ID No. 4).
11. 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), wherein the modified oncolytic poxvirus generates an increased number of comet tail type plaques in a viral plaque forming assay, compared to an otherwise identical oncolytic virus that does not comprise the at least two non-naturally occurring mutations.
12. 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.
13. 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.
14. 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.
15. The modified oncolytic poxvirus of claim 10, wherein the neutralizing antibody is an anti-L1 NR-45114 antibody or an anti-VIG antibody.
16. The modified oncolytic poxvirus of claim 9 or 10, wherein the A34R protein or the fragment thereof further comprises a mutation at position Lys151 of the wild-type A34R protein (SEQ ID No. 4).
17. The modified oncolytic poxvirus of claim 1, wherein the amino acid residues that are positively charged at pH 5 are lysine residues.
18. The modified oncolytic poxvirus of any one of claims 5-12 and 16-17, wherein 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.

19. The modified oncolytic poxvirus of any one of claims 5-12 and 16-17, wherein 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.
20. The modified oncolytic poxvirus of any one of claims 5-8, 11-12, and 17-19, wherein at least one of the two non-naturally occurring mutations is at position Lys119 of the wild-type A34R protein (SEQ ID No. 4).
21. The modified oncolytic poxvirus of any one of claims 9 and 18-19, wherein the non-naturally occurring mutation is at position Lys119 of the wild-type A34R protein (SEQ ID No. 4).
22. The modified oncolytic poxvirus of claim 10 or 21, wherein the mutation at position Lys119 of the wild-type A34R protein (SEQ ID No. 4) is Lys119Glu.
23. The modified oncolytic poxvirus of any one of claims 5-8, 11-12, 17-19, and 20-22, wherein at least one of the two non-naturally occurring mutations is at position Lys151 of the wild-type A34R protein (SEQ ID No. 4).
24. The modified oncolytic poxvirus of claim 23, wherein the mutation at position Lys151 of the wild-type A34R protein (SEQ ID No. 4) is Lys151Glu.
25. A modified oncolytic poxvirus that expresses an A34R protein comprising mutations Lys119Glu and Lys151Glu
26. The modified oncolytic poxvirus of any one of claims 19-23, wherein positions 305-307 of SEQ ID No. 3 comprises nucleotides GAA or GAG.
27. The modified oncolytic poxvirus of any one of claims 19-23 and 26, wherein positions 451-453 of SEQ ID No. 3 comprises nucleotides GAA or GAG.
28. The modified oncolytic poxvirus of any one of claims 5-8, 11-12, and 17-27, wherein 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.
29. The modified oncolytic virus of any one of claims 9-10, 16, and 21-22, wherein 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.
30. The modified oncolytic poxvirus of any of one of claims 1-29, further comprising an exogenous nucleic acid that codes for at least one of: a therapeutic protein or a diagnostic protein.

31. The modified oncolytic poxvirus of claim 30, wherein 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-R α , LIGHT, ITAC, fractalkine, CCL5, N1L, an immune checkpoint modulator, a metabolic modulating protein, or any combinations thereof.
32. The modified oncolytic poxvirus of claim 31, comprising the exogenous nucleic acid that codes for a chemokine receptor, wherein the chemokine receptor comprises at least one of CXCR4 and CCR2.
33. The modified oncolytic poxvirus of claim 31 or 32 comprising the exogenous nucleic acid that codes for the membrane associated protein.
34. The modified oncolytic poxvirus of claim 33, wherein the membrane associated protein comprises a membrane associated hyaluronidase.
35. The modified oncolytic poxvirus of claim 34, wherein the membrane associated hyaluronidase comprises PH-20.
36. The modified oncolytic poxvirus of claim 35, wherein the PH-20 is GPI-anchored.
37. The modified oncolytic virus of any one of claims 31 - 36, comprising the exogenous nucleic acid that codes for the microbial protein that is capable of degrading hyaluronan, wherein the microbial protein comprises a secreted hyaluronidase.
38. The modified oncolytic poxvirus of claim 37, wherein the secreted hyaluronidase comprises at least one of HysA, lin, sko, and rv, or any combinations thereof.
39. The modified oncolytic poxvirus of any one of claims 31-38, comprising the exogenous nucleic acid that codes for the microbial protein.
40. The modified oncolytic poxvirus of claim 43, wherein the microbial protein comprises HysA.
41. The modified oncolytic poxvirus of any one of claims 1-40, further comprising a modification in the genome of the virus, wherein the modification comprises a mutation or a deletion of the B5R gene.
42. The modified oncolytic poxvirus of claim 41, comprising 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.
43. The modified oncolytic poxvirus of any one of claims 1-42, further comprising 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.

44. The modified oncolytic poxvirus of claim 43, comprising the mutation or deletion of viral gene A52R.

45. The modified oncolytic poxvirus of any one of claims 31-44, comprising (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.

46. The modified oncolytic poxvirus of any one of claims 1-45, wherein the virus is suitable for systemic delivery.

47. The modified oncolytic poxvirus of any one of claims 1-46, wherein the virus is capable of immune evasion.

48. The modified oncolytic poxvirus of claim 46 or 47, wherein the systemic delivery comprises oral administration, parenteral administration, intranasal administration, sublingual administration, rectal administration, transdermal administration, or any combinations thereof.

49. The modified oncolytic poxvirus of claim 48, wherein the parenteral administration comprises an intravenous injection.

50. The modified oncolytic poxvirus of any one of claims 1-49, wherein the virus is suitable for intratumoral delivery.

51. The modified oncolytic poxvirus of any one of claims 1-50, wherein the poxvirus is a vaccinia virus.

52. 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.

53. The process of claim 52, wherein the mammalian cells comprise HeLa cells, 293 cells, A549 cells, or Vero cells.

54. A kit, comprising: an oncolytic poxvirus according to any one of claims 1-53, a container; and instructions for administering said oncolytic virus to a subject to treat a disorder associated with pathological angiogenesis.
55. A method of treating a tumor, the method comprising administering to a subject a therapeutically effective amount of an oncolytic poxvirus according to any one of claims 1-54.
56. 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.
57. The method of claim 56, wherein the patient-derived leukocyte cells comprise macrophages.
58. The method of claim 56, wherein the patient-derived leukocyte cells comprise tumor-targeted T cells.
59. The method of claim 56, wherein 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.
60. The method of any one of claims 56-59, further comprising harvesting the EEV particles from the tumor microenvironment and intravenously administering the EEV particles to the subject.
61. The method of any one of claims 56-60, wherein the modified oncolytic poxvirus is a modified oncolytic vaccinia virus.
62. 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).
63. The process of claim 62, wherein the modified oncolytic poxvirus is a modified oncolytic vaccinia virus.
64. A method of treating a cancer, the method comprising administering to a patient a modified oncolytic virus 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).

65. The method of claim 64, wherein 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.
66. A method of treating a tumor, the method comprising administering to a patient a modified oncolytic virus 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).
67. The method of claim 66, wherein 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.
68. The method of any one of claims 64-67, wherein the administering is via an intratumoral injection, an intravenous injection, or a combination thereof.
69. The method of any one of claims 55-61, wherein the administering is via an intratumoral injection, an intravenous injection, or a combination thereof.
70. The method of claim 68 or 69, wherein the method further comprises administering a further therapy, in combination with the oncolytic poxvirus, wherein the further therapy comprises 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.
71. The method of claim 70, wherein 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.