## CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant oncolytic virus, comprising:

an inactivating mutation of B2R;

a heterologous nucleic acid encoding interferon regulatory factor 3 (IRF3); and

at least one heterologous nucleic acid encoding one or more cytokine and/or chemokine.

2. The recombinant oncolytic virus of claim 1, wherein the at least one heterologous nucleic acid encoding one or more cytokine and/or chemokine comprises:

a heterologous nucleic acid encoding chemokine ligand 9 (CXCL9) and/or IL-12; or

a heterologous nucleic acid encoding CXCL9 and a heterologous nucleic acid encoding IL-12.

3. The recombinant oncolytic virus of claim 1 or claim 2, further comprising at least one heterologous nucleic acid encoding one or more heterologous gene product.

4. A recombinant oncolytic virus, comprising:

an inactivating mutation of at least one viral gene; and

at least one heterologous nucleic acid encoding one or more heterologous gene product, wherein the one or more heterologous gene product is or comprises an immune modulating protein, a complement inhibitor, a T cell or NK cell evader, an anti-angiogenic protein, an interferon regulatory factor, or an apoptosis inducible protein, or a combination of any of the foregoing.

5. The recombinant oncolytic virus of claim 4, wherein the at least one viral gene comprises one or more viral genes selected from the group consisting of hemagglutinin (HA), J2R (thymidine kinase), F14.5L, A56R (hemagglutinin), B2R, vaccinia growth factor (VGF), A35R, A49R, A55R, B14R, C4L, C6L, C16L, NIL/N2L, E2L/E3L, K1L/K2L, K7L, superoxide dismutase locus, 7.5K, C2L-F3L, C4L-F1L, C7-K1L, B13R+B14R, A26L, and I4L, and any combination thereof.

6. The recombinant oncolytic virus of claim 4 or claim 5, wherein the inactivating mutation of one or more of the at least one viral gene is, independently, by:

insertion of at least one of the at least one heterologous nucleic acid encoding one or more heterologous gene product into a gene loci in the genome of the virus;

a deletion of all or a portion of the at least one viral gene; and/or

one or more nucleic acid substitutions in the at least one viral gene.

7. The recombinant oncolytic virus of any one of claims 4-6, wherein the at least one viral gene comprises J2R.

8. A recombinant oncolytic virus, comprising at least one heterologous nucleic acid encoding one or more heterologous gene product, wherein the one or more heterologous gene product is or comprises a complement inhibitor, a T cell or NK cell evader, an immune modulating protein, an anti-angiogenic protein, an interferon regulatory factor, an apoptosis inducible protein, or a combination of any of the foregoing.

9. A recombinant oncolytic virus, comprising a nucleic acid genome that is modified from a parental vaccinia virus genome having at least 99% sequence identity with the nucleic acid sequence set forth in SEQ ID NO: 1, wherein the nucleic acid genome of the recombinant oncolytic virus comprises at least one heterologous nucleic acid encoding one or more heterologous gene product inserted in the genome.

10. The recombinant oncolytic virus of any one of claims 4-9, wherein the at least one heterologous nucleic acid encoding one or more heterologous gene product comprises one or more heterologous nucleic acid each encoding one or more immune modulating proteins.

11. The recombinant oncolytic virus of claim 10, wherein the one or more immune modulating proteins comprises one or more immune stimulating proteins, one or more immune stimulating proteins, one or more cytokines and/or chemokines, or one or more interferon regulatory factors, optionally wherein the one or more immune modulating proteins comprises one or more immune modulating proteins selected from the group consisting of LIGHT, IRF3, IL-2, IL-12, and CXCL9.

12. The recombinant oncolytic virus of any one of claims 4-11, wherein the at least one heterologous nucleic acid encoding one or more heterologous gene product comprises at least one heterologous nucleic acid encoding one or more cytokine and/or chemokine.

13. The recombinant oncolytic virus of any one of claims 1-3 and 12, wherein the at least one heterologous nucleic acid encoding one or more cytokine and/or chemokine comprises a heterologous nucleic acid encoding IL-2.

14. The recombinant oncolytic virus of claim 13, wherein the IL-2 is an IL-2 superkine.

15. The recombinant oncolytic ~~vaccinia~~ virus of any one of claims 1-9, wherein the at least one heterologous nucleic acid encoding one or more heterologous gene product encodes one or more

heterologous gene product selected from the group consisting of a complement inhibitor, a T cell or NK cell evader, an immune stimulating protein, an anti-angiogenic protein, an interferon regulatory factor, an apoptosis inducible protein, or a combination of any of the foregoing.

16. The recombinant oncolytic virus of any of claims 1-3 and 13-15, wherein the inactivating mutation of B2R is characterized by insertion of the heterologous nucleic acid encoding IRF3 and/or at least one of the at least one heterologous nucleic acid encoding one or more cytokine and/or chemokine into the B2R gene loci.

17. The recombinant oncolytic virus of any one of claims 4-15, comprising an inactivating mutation of B2R.

18. The recombinant oncolytic virus of any one of claims 4-15 and 17, wherein the at least one heterologous nucleic acid encoding one or more heterologous gene product comprises a heterologous nucleic acid encoding interferon regulatory factor 3 (IRF3).

19. The recombinant oncolytic virus of any of claims 4-15, comprising an inactivating mutation of B2R, wherein the inactivating mutation of B2R is characterized by insertion of the heterologous nucleic acid encoding IRF3 and/or at least one of the at least one heterologous nucleic acid encoding one or more cytokine and/or chemokine into the B2R gene loci.

20. The recombinant oncolytic virus of any one of claims 1-19, wherein the nucleic acid genome of the recombinant oncolytic virus is modified from a parental vaccinia virus that has a nucleic acid genome that has at least 99% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 1, and wherein the nucleic acid genome of the parental virus is characterized by one or more of:

(i) a guanine (G) at the position corresponding to position 7770 of SEQ ID NO: 1;

(ii) a thymine (T) at the position corresponding to position 15261 of SEQ ID NO: 1;

(iii) a G at the position corresponding to position 32136 of SEQ ID NO: 1;

(iv) a G at the position corresponding to position 49455 of SEQ ID NO: 1;

(v) a cytosine (C) at the position corresponding to position 92969 of SEQ ID NO: 1;

(vi) the nucleic acid sequence CACTTATATAT at the positions corresponding to positions 106870 to 106880 of SEQ ID NO: 1;

(vii) the nucleic acid sequence GTTTTCATTA at the positions corresponding to positions 111267 to 111276 of SEQ ID NO: 1;

(viii) an adenine (A) at the position corresponding to position 162715 of SEQ ID NO: 1;

(ix) the nucleic acid sequence TACAGACACC at the positions corresponding to positions 165844 to 185853 of SEQ ID NO: 1; and

(x) a C at the position corresponding to position 187805 of SEQ ID NO: 1.

21. The recombinant oncolytic virus of any one of claims 1-20, wherein the nucleic acid genome of the recombinant oncolytic virus has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 1.

22. The recombinant oncolytic virus of any one of claims 1-3 and 13-15, wherein:

the heterologous nucleic acid encoding IRF3 is inserted into the J2R (thymidine kinase) gene locus in the genome of the virus; and

the at least one heterologous nucleic acid encoding one or more cytokine and/or chemokine comprises a heterologous nucleic acid encoding CXCL9 and IL-12, wherein the heterologous nucleic acid encoding CXCL9 and IL-12 is inserted into the A56R gene locus in the genome of the virus.

23. The recombinant oncolytic virus of claim 17, wherein:

the heterologous nucleic acid encoding IRF3 is inserted into the J2R (thymidine kinase) gene locus in the genome of the virus;

the at least one heterologous nucleic acid encoding one or more heterologous gene product comprises at least one heterologous nucleic acid encoding one or more cytokine and/or chemokine; and

the at least one heterologous nucleic acid encoding one or more cytokine and/or chemokine comprises a heterologous nucleic acid encoding CXCL9 and IL-12, wherein the heterologous nucleic acid encoding CXCL9 and IL-12 is inserted into the A56R gene locus in the genome of the virus.

24. The recombinant oncolytic virus of any one of claims 1-15, 22, and 23, wherein:

the nucleic acid genome of the recombinant oncolytic virus comprises the nucleic acid sequence of SEQ ID NO: 85, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 85, or

the nucleic acid genome of the recombinant oncolytic virus comprises the nucleic acid sequence of SEQ ID NO: 92, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 92.

25. The recombinant oncolytic ~~vaccinia~~ virus of any one of claims 1-3 and 13-15, wherein the heterologous nucleic acid encoding IRF3 is inserted into the B2R (viral cGAMP-specific nuclease) gene locus in the genome of the virus; and

the at least one heterologous nucleic acid encoding one or more cytokine and/or chemokine comprises a heterologous nucleic acid encoding CXCL9 and IL-12, wherein the heterologous nucleic acid encoding CXCL9 and IL-12 is inserted into the A56R gene locus in the genome of the virus.

26. The recombinant oncolytic virus of claim 17, wherein the heterologous nucleic acid encoding IRF3 is inserted into the B2R (viral cGAMP-specific nuclease) gene locus in the genome of the virus;

the at least one heterologous nucleic acid encoding one or more heterologous gene product comprises at least one heterologous nucleic acid encoding one or more cytokine and/or chemokine; and

the at least one heterologous nucleic acid encoding one or more cytokine and/or chemokine comprises a heterologous nucleic acid encoding CXCL9 and IL-12, wherein the heterologous nucleic acid encoding CXCL9 and IL-12 is inserted into the A56R gene locus in the genome of the virus.

27. The recombinant oncolytic virus of any one of claims 1-15, 25, and 26, wherein:

the nucleic acid genome of the recombinant oncolytic virus comprises the nucleic acid sequence of SEQ ID NO: 86, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 86;

the nucleic acid genome of the recombinant oncolytic virus comprises the nucleic acid sequence of SEQ ID NO: 88, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 88; or

the nucleic acid genome of the recombinant oncolytic virus comprises the nucleic acid sequence of SEQ ID NO: 90, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 90.

28. The recombinant oncolytic virus of any one of claims 3-27, wherein the at least one heterologous nucleic acid encoding one or more heterologous gene product comprises a heterologous nucleic acid encoding an apoptosis-inducible protein.

29. The recombinant oncolytic virus of claim 28, wherein the apoptosis-inducible protein comprises a proapoptotic molecule fused with an FKBP variant that is able to bind a chemical inducer of dimerization (CID).

30. The recombinant oncolytic virus of claim 28 or claim 29, wherein the apoptosis-inducible protein is an inducible death effector domain (iDED).

31. The recombinant oncolytic virus of claim 30, wherein the heterologous nucleic acid encoding an iDED is inserted into or in place of the J2R gene locus in the genome of the virus.

32. The recombinant oncolytic virus of any one of claims 3-9 and 28-31, wherein:

the nucleic acid genome of the recombinant oncolytic virus comprises the nucleic acid sequence of SEQ ID NO: 86, or a nucleic acid sequence that has at least 95%~~, 96%, 97%, 98%, or 99%~~ sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 86; or

the nucleic acid genome of the recombinant oncolytic virus comprises the nucleic acid sequence set forth in SEQ ID NO: 8, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 8.

33. The recombinant oncolytic virus of any one of claims 3-32, wherein the at least one heterologous nucleic acid encoding one or more heterologous gene product comprises a heterologous nucleic acid encoding one or more T cell or NK cell evader proteins.

34. The recombinant oncolytic virus of claim 33, wherein the one or more T cell or NK cell evader proteins comprises a set of proteins encoded by Cowpox virus ORFs 012, 203 and 018 (CPXV012-203-018).

35. The recombinant oncolytic virus of any one of claims 3-34, wherein the at least one heterologous nucleic acid encoding one or more heterologous gene product comprises a heterologous nucleic acid encoding a complement inhibitor.

36. The recombinant oncolytic virus of claim 35, wherein the complement inhibitor is *Borrelia burgdorferi* complement regulatory-acquiring surface protein-2 (CRASP-2).

37. The recombinant oncolytic virus of claim 36, wherein the heterologous nucleic acid encoding CRASP-2 is fused with a viral membrane gene, optionally F14.5L, to produce a fusion gene encoding a fusion protein.

38. The recombinant oncolytic virus of claim 37, wherein the heterologous nucleic acid encoding CRASP-2 fused with the viral membrane gene, optionally F14.5L, is inserted into J2R.

39. The recombinant oncolytic virus of any one of claims 1-9 and 35-38, wherein:

the nucleic acid genome of the recombinant oncolytic virus comprises the nucleic acid sequence of SEQ ID NO: 90, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 90; or

the nucleic acid genome of the recombinant oncolytic virus comprises the nucleic acid sequence of SEQ ID NO: 5, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 5.

40. The recombinant oncolytic virus of any one of claims 3-39, wherein the at least one heterologous nucleic acid encoding one or more heterologous gene product comprises a heterologous nucleic acid encoding an immune stimulating protein, and/or a heterologous nucleic acid encoding one or more anti-angiogenic protein.

41. The recombinant oncolytic virus of claim 40, wherein:

the immune stimulating protein is recombinant LIGHT; and/or

the one or more anti-angiogenic protein comprises:

a VEGF inhibitor, an angiopoietin inhibitor, versikine, or a fusion protein of any two or more of the foregoing, or

a bispecific anti-VEGF/anti-Ang2 antibody.

42. The recombinant oncolytic virus of any one of claims 1-9, 40, and 41, wherein the nucleic acid genome of the recombinant oncolytic virus comprises the nucleic acid sequence of SEQ ID NO: 13 or 88, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 13 or 88.

43. The recombinant oncolytic virus of any one of claims 3-42, wherein the at least one heterologous nucleic acid encoding one or more heterologous gene product comprises one or more heterologous nucleic acid each encoding one or more therapeutic agent or diagnostic agent.

44. An isolated clonal vaccinia virus (VACV) strain comprising a nucleic acid genome that has at least 95% sequence identity with the nucleic acid sequence set forth in SEQ ID NO: 1 and wherein the nucleic acid genome is characterized by one or more of:

(i) a variant 017 open reading frame (ORF) encoding an amino acid sequence that has at least 95% sequence identity to SEQ ID NO: 57 and comprises a polar uncharged amino acid at position 66, optionally a threonine (T) at position 66;

(ii) a variant 038 (K5L) ORF comprising a nucleotide insertion to effect a frameshift mutation, wherein the 038 (K5L) gene product is altered;

(iii) a variant 059 (E2L) ORF encoding an amino acid sequence that is at least 95% sequence identity to SEQ ID NO:60 and comprises a hydrophobic amino acid other than leucine at position 419, optionally a phenylalanine (F) at position 419;

(iv) a variant 104 (H4L) ORF encoding an amino acid sequence having at least 95% sequence identity to SEQ ID NO:61 and comprises a negatively charged amino acid at position 591, optionally aspartic acid (D) at position 591; and

(v) a variant 182 (A56R) ORF comprising deletion of two nucleotides to effect a frameshift mutation, wherein the 182 (A56R) ORF gene product is altered.

45. An isolated clonal vaccinia virus (VACV) strain comprising a nucleic acid genome that has at least 95% sequence identity with the nucleic acid sequence set forth in SEQ ID NO: 1, and wherein the nucleic acid genome is characterized by one or more of:

(i) a guanine (G) at the position corresponding to position 7770 of SEQ ID NO: 1;

(ii) a thymine (T) at the position corresponding to position 15261 of SEQ ID NO: 1;

(iii) a G at the position corresponding to position 32136 of SEQ ID NO: 1;

(iv) a G at the position corresponding to position 49455 of SEQ ID NO: 1;

(v) a cytosine (C) at the position corresponding to position 92969 of SEQ ID NO: 1;

(vi) the nucleic acid sequence CACTTATATAT at the positions corresponding to positions 106870 to 106880 of SEQ ID NO: 1;

(vii) the nucleic acid sequence GTTTTCATTA at the positions corresponding to positions 111267 to 111276 of SEQ ID NO: 1;

(viii) an adenine (A) at the position corresponding to position 162715 of SEQ ID NO: 1;

(ix) the nucleic acid sequence TACAGACACC at the positions corresponding to positions 165844 to 185853 of SEQ ID NO: 1; and

(x) a C at the position corresponding to position 187805 of SEQ ID NO: 1.

46. A VACV preparation comprising the isolated clonal VACV strain of claim 44 or claim 45.

47. A VACV preparation comprising the recombinant oncolytic virus of any one of claims 1-43, wherein the recombinant oncolytic virus is a recombinant oncolytic vaccinia virus.

48. A recombinant oncolytic virus preparation comprising the recombinant oncolytic virus of any one of claims 1-43.

49. A pharmaceutical composition comprising the isolated VACV clonal strain of claim 44 or claim 45.

50. A pharmaceutical composition comprising the VACV preparation of claim 46 or claim 47.

51. A pharmaceutical composition comprising the recombinant oncolytic virus of any one of claims 1-43.

52. A recombinant vaccinia virus (VACV) strain comprising a nucleic acid genome of the isolated VACV clonal strain of claim 44 or claim 45 that comprises an inactivating mutation in at least one viral gene.

53. The recombinant VACV strain of claim 52, wherein the viral gene is selected from the group consisting of hemagglutinin (HA), J2R (thymidine kinase), F14.5L, A56R (hemagglutinin), B2R, vaccinia growth factor (VGF), A35R, A49R, A55R, B14R, C4L, C6L, C16L, NIL/N2L, E2L/E3L, K1L/K2L, K7L, superoxide dismutase locus, 7.5K, C2L-F3L, C4L-F1L, C7-K1L, B13R+B14R, A26L and I4L.

54. The recombinant VACV strain of claim 52 or claim 53, further comprising at least one heterologous nucleic acid encoding one or more heterologous gene product, wherein the one or more heterologous gene product is or comprises an immune modulating protein, a complement inhibitor, a T cell or NK cell evader, an anti-angiogenic protein, an interferon regulatory factor, or an apoptosis inducible protein, or a combination of any of the foregoing.

55. The recombinant VACV strain of any one of claims 52-54, wherein the nucleic acid genome of the recombinant oncolytic virus comprises a nucleic acid sequence set forth in any one of SEQ ID NOs: 5, 8, 13, 48, 80, 82, and 84-93, or a nucleic acid sequence that has at least 95% sequence identity to a nucleic acid sequence set forth in any one of SEQ ID NOs: 5, 8, 13, 48, 80, 82, and 84-93.

56. A nucleic acid comprising a genome of the recombinant oncolytic virus of any one of claims 1-43, or the isolated VACV clonal strain of claim 44 or claim 45.

57. A recombinant oncolytic virus comprising the nucleic acid of claim 56.

58. A pharmaceutical composition comprising the recombinant VACV strain of any of claims 52-55.

59. A pharmaceutical composition comprising the recombinant oncolytic virus of any one of claims 1-43, the isolated clonal VACV strain of claim 44 or claim 45, or the recombinant VACV strain of any one of claims 52-55 for the treatment of a proliferative disorder in a subject.

60. A pharmaceutical composition of any one of claims 49-51 and 58 for the treatment of a proliferative disorder in a subject.

61. Use of the recombinant oncolytic virus of any one of claims 1-43, the isolated clonal VACV strain of claim 44 or claim 45, the recombinant VACV strain of any one of claims 52-55, or the pharmaceutical composition of any one of claims 49-51 and 58 for the preparation of a medicament for treating a proliferative disorder in a subject.

62. A pharmaceutical composition comprising AP1903 (Rimiducid) for inhibiting virus replication, wherein the inhibiting comprises contacting cells infected with a recombinant oncolytic virus with the pharmaceutical composition, wherein the recombinant oncolytic virus comprises a heterologous nucleic acid encoding an apoptosis inducible protein.

63. Use of a recombinant oncolytic virus for the preparation of a medicament for inhibiting virus replication by contacting cells infected with the recombinant oncolytic virus with AP1903 (Rimiducid), wherein the recombinant oncolytic virus comprises a heterologous nucleic acid encoding an apoptosis inducible protein.

64. A pharmaceutical composition comprising a recombinant oncolytic vaccinia virus for the treatment of a proliferative disorder in a subject, wherein the nucleic acid genome of the recombinant oncolytic vaccinia virus comprises:

the nucleic acid sequence of SEQ ID NO: 8, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 8; or

the nucleic acid sequence of SEQ ID NO: 85, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 85; or

the nucleic acid sequence of SEQ ID NO: 86, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 86; or

the nucleic acid sequence of SEQ ID NO: 88, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 88; or

the nucleic acid sequence of SEQ ID NO: 90, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 90; or

the nucleic acid sequence of SEQ ID NO: 92, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 92; or

the nucleic acid sequence of SEQ ID NO: 5, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 5; or

the nucleic acid sequence of SEQ ID NO: 13, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 13.

65. Use of a recombinant oncolytic vaccinia virus for preparing a medicament for treating a proliferative disorder in a subject, wherein the nucleic acid genome of the recombinant oncolytic vaccinia virus comprises:

the nucleic acid sequence of SEQ ID NO: 8, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 8;

the nucleic acid sequence of SEQ ID NO: 85, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 85; or

the nucleic acid sequence of SEQ ID NO: 86, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 86; or

the nucleic acid sequence of SEQ ID NO: 88, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 88; or

the nucleic acid sequence of SEQ ID NO: 90, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 90; or

the nucleic acid sequence of SEQ ID NO: 92, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 92; or

the nucleic acid sequence of SEQ ID NO: 5, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 5; or

the nucleic acid sequence of SEQ ID NO: 13, or a nucleic acid sequence that has at least 95% sequence identity to the nucleic acid sequence set forth in SEQ ID NO: 13.