

CLAIMS**WHAT IS CLAIMED IS:**

1. A myxoma virus (MYXV) having enhanced anti-cancer activity, wherein the myxoma virus is genetically engineered to attenuate an activity or expression level of its M153 protein.
2. The MYXV of claim 1, wherein the activity or the expression level of the M153 protein is attenuated at least 80%.
3. The MYXV of claim 1 or claim 2, wherein the MYXV is engineered to introduce a mutation in a nucleic acid encoding the M153 protein, wherein the mutation comprises an insertion, deletion, or substitution mutation.
4. The MYXV of claim 3, wherein the mutation enhances cell immune response activity in relation to a wild-type M153 protein.
5. The MYXV of claim 1, wherein at least a portion of a nucleic acid encoding the M153 protein in MYXV genome is knocked out.
6. The MYXV of claim 1, wherein the MYXV comprises an inhibitory molecule targeting M153 transcript that thereby attenuates the M153 protein expression.
7. The MYXV of claim 6, wherein the inhibitory molecule is an inhibitory RNA.
8. The MYXV of claim 7, wherein the inhibitory RNA comprises dsRNA, siRNA, antisense RNA, or miRNA.
9. The MYXV of any one of claims 1-8, further comprising a nucleic acid encoding a non-viral molecule.
10. The MYXV of claim 9, wherein the non-viral molecule is tumor necrosis factor alpha (TNF α).
11. The MYXV of claim 10, wherein the TNF α is human TNF α .
12. The MYXV of claim 10 or 11, wherein the TNF α is a soluble peptide.
13. The MYXV of any of claims 10-12, wherein the TNF α is a membrane- or surface-bound peptide.
14. The MYXV of any of claims 10-13, wherein the TNF α further enhances the anti-cancer activity of the MYXV by activating anti-tumor immune cells or inducing cancer cell death.
15. The MYXV of claim 9, wherein the non-viral molecule is interleukin-12 (IL-12).
16. The MYXV of claim 15, wherein the IL-12 is human IL-12.
17. The MYXV of claim 15 or claim 16, wherein the IL-12 is a soluble peptide.
18. The MYXV of any one of claims 15-17, wherein the IL-12 is a membrane- or surface-bound peptide.

19. The MYXV of any of claims 15-18, wherein the IL-12 further enhances the anti-cancer activity of the MYXV by promoting immune cell differentiation or eliciting immune cell cytotoxicity.
20. The MYXV of claim 9, wherein the non-viral molecule is decorin.
21. The MYXV of claim 20, wherein the decorin is human decorin.
22. The MYXV of claim 20 or 21, wherein the decorin is a soluble peptide.
23. The MYXV of any of claims 20-22, wherein the decorin is a membrane- or surface-bound peptide.
24. The MYXV of any of claims 20-23, wherein the decorin further enhances the anti-cancer activity of the MYXV by blocking or decreasing TGF- β signaling.
25. The MYXV of claim 9, wherein the nucleic acid encodes at least two molecules selected from a group consisting of TNF α , IL-12, and decorin.
26. The MYXV of claim 9, wherein the nucleic acid encodes TNF α , IL-12, and decorin.
27. The MYXV of any of claims 1-24, wherein the MYXV is derived from a *Lausanne* strain.
28. A composition comprising a MYXV of any of claims 1-27, and a pharmaceutically acceptable carrier or excipient.
29. The composition of claim 28, formulated for systemic administration.
30. The composition of claim 28, formulated for local administration.
31. The composition of any one of claims 28-30, formulated for parenteral administration.
32. A composition comprising a plurality of cells treated *ex vivo* by the MYXV of any one of claims 1-27, wherein the plurality of cells comprises peripheral blood mononuclear cells (PBMCs), bone marrow (BM) cells, or a combination thereof.
33. A method of inhibiting, alleviating, or preventing a cancer in a subject in need thereof, comprising administering to the subject the MYXV of any one of claim 1-27 or the composition of any one of claims 28-32.
34. The method of claim 33, wherein the subject has, is suspected of having, or is at risk of having the cancer, and wherein the method further comprises selecting the subject.
35. The method of claim 33 or claim 34, wherein the subject is a human.
36. The method of any one of the claims 33-35, wherein the administration is systemic administration.
37. The method of any one of the claims 33-36, wherein the administration reduces cancer cell viability, or activates immunogenic cell death in the cancer.

38. The method of any one of the claims 33-37, wherein the administration improves the subject's survival.

39. The method of any one of the claims 33-38, wherein the cancer comprises a solid tumor.

40. The method of any one of the claims 33-38, wherein the cancer is an osteosarcoma, triple negative breast cancer, or melanoma.

41. The method of any one of the claims 33-40, wherein the cancer has metastasized to a location in the subject.

42. The method of claim 41, wherein the location comprises a lung, a brain, a liver and/or a lymph node of the subject.

43. The method of any one of the claims 33-42, further comprising administering to the subject an additional therapeutic agent.

44. The method of claim 43, wherein the additional therapeutic agent is an immune checkpoint modulator.

45. The method of claim 43 or claim 44, wherein the additional therapeutic agent is administered to the subject before administering the composition.

46. The method of claim 43 or claim 44, wherein the additional therapeutic agent is administered to the subject after administering the composition.

47. The method of claim 43 or claim 44, wherein the additional therapeutic agent is administered to the subject as a combination with the composition.

48. The method of any one of claims 33-47, wherein the composition comprising the plurality of cells is administered to the subject, wherein the plurality of cells comprises cells that are autologous to the subject.

49. The method of any one of claims 33-47, wherein the composition comprising the plurality of cells is administered to the subject, wherein the plurality of cells comprises cells that are allogenic to the subject.

50. A recombinant nucleic acid comprising at least a portion of MYXV genome, wherein the MYXV genome is modified to reduce expression of M153 gene.

51. The recombinant nucleic acid of claim 50, wherein the recombinant nucleic acid comprises DNA.

52. The recombinant nucleic acid of claim 50 or claim 51, wherein the portion of MYXV genome is modified to knock out at least a portion of the M153 gene in the portion of MYXV genome.

53. The recombinant nucleic acid of any of claims 50-52, wherein the recombinant MYXV genome comprises a first nucleic acid encoding TNF α .

54. The recombinant nucleic acid of claim 53, wherein the TNF α is human TNF α .
55. The recombinant nucleic acid of claim 53 or claim 54, wherein the first nucleic acid replaces or is adjacent to an M135R gene of the MYXV genome.
56. The recombinant nucleic acid of any of claims 53-55, wherein first nucleic acid is inserted between an M135R gene and an M136R gene of the MYXV genome.
57. The recombinant nucleic acid of any of claims 53-56, wherein expression of the first nucleic acid is driven by a poxvirus synthetic early/late (sE/L) promoter.
58. The recombinant nucleic acid of any of claims 50-57, wherein the recombinant MYXV genome comprises a second nucleic acid encoding interleukin-12 subunit alpha (IL-12 α).
59. The recombinant nucleic acid of claim 58, wherein the IL-12 α is human IL-12 α .
60. The recombinant nucleic acid of claim 58 or claim 59, wherein expression of IL-12 α is driven by an internal ribosome entry site (IRES).
61. The recombinant nucleic acid of any of claims 58-60, wherein the second nucleic acid IL-12 α disrupts the expression of the M153 gene of the MYXV genome.
62. The recombinant nucleic acid of any of claims 50-61, wherein the recombinant MYXV genome comprises a third nucleic acid encoding an interleukin-12 subunit beta (IL-12 β).
63. The recombinant nucleic acid of claim 62, wherein the IL-12 β is human IL-12 β .
64. The recombinant nucleic acid of claim 62 or claim 63, wherein expression of the third nucleic acid is driven by an sE/L promoter.
65. The recombinant nucleic acid of any of claims 62-64, wherein the third nucleic acid disrupts the expression of the M153 gene of the MYXV genome.
66. The recombinant nucleic acid of any of claims 50-65, wherein the recombinant MYXV genome comprises a fourth nucleic acid encoding decorin.
67. The recombinant nucleic acid of claim 66, wherein the decorin is human decorin.
68. The recombinant nucleic acid of claim 66 or claim 67, wherein expression of fourth nucleic acid is driven by an sE/L promoter.
69. The recombinant nucleic acid of any of claims 66-68, wherein the fourth nucleic acid disrupts expression of the M153 gene of the MYXV genome.
70. The recombinant nucleic acid of any of claims 50-69, further comprising a fifth nucleic acid encoding a reporter tag.
71. The recombinant nucleic acid of claim 70, wherein the reporter tag comprises a green fluorescent protein (GFP).
72. The recombinant nucleic acid of claim 70 or claim 71, wherein expression of the fifth nucleic acid is driven by an sE/L promoter.

73. The recombinant nucleic acid of claim 70, further comprising a sixth nucleic acid encoding a second reporter tag.

74. The recombinant nucleic acid of claim 73, wherein the second reporter tag comprises a red fluorescent protein (RFP).

75. The recombinant nucleic acid of claim 73 or claim 74, wherein expression of the sixth nucleic acid is driven by a poxvirus P11 late promoter.

76. The recombinant nucleic acid of any of claims 50-75, wherein the recombinant nucleic acid comprises a vMyx-hTNFa cassette, optionally comprising GFP.

77. The recombinant nucleic acid of any of claims 50-76, wherein the recombinant nucleic acid comprises an hDecorin-hIL-12 cassette, optionally comprising dsRed.

78. The recombinant nucleic acid of any of claims 50-77, wherein the recombinant nucleic acid comprises or consists of a vMyx-hTNFa-hDecorin-hIL-12-M153KO (vMyx-Triple) cassette, optionally comprising dsRed and/or GFP.

79. A recombinant MYXV comprising the recombinant nucleic acid of any of claims 50-78.

80. A method of inhibiting, alleviating, or preventing a cancer in a subject in need thereof, comprising administering to the subject the recombinant nucleic acid of any one of claims 50-79 or the recombinant MYXV of claim 79.

81. The method of claim 80, wherein the subject has, is suspected of having, or is at risk of having the cancer, and wherein the method further comprises selecting the subject.

82. The method of claims 80 or 81, wherein the subject is a human.

83. The method of any one of claims 80-82, wherein the administration is systemic administration.

84. The method of any one of the claims 80-83, wherein the administration reduces cell viability, or activates immunogenic cell death in the cancer.

85. The method of any one of the claims 35-49 or any one of claims 80-84, wherein the administration is performed in a dose and a schedule effective to increase expression of at least two cytokines in PBMCs of the subject.

86. The method of any one of the claims 35-49 or any one of claims 80-84, wherein the administration is performed in a dose and a schedule effective to increase expression of at least two cytokines in cancer cells in the subject.

87. The method of claim 85 or claim 86, wherein the at least two cytokines comprise IFN- γ , IL-2, IL-6, IL-10, IL-12, or TNF- α .

88. The method of any one of the claims 35-49 or any one of claims 80-87, wherein the administration is performed in a dose and a schedule effective to reduce volume of the cancer by at least 10%.

89. The method of any one of the claims 35-49 or any one of claims 80-87, wherein the administration is performed in a dose and a schedule effective to reduce growth of the cancer by at least 10%.

90. The MYXV of claim 15, wherein the IL-12 comprises an IL-12 α subunit and an IL-12 β subunit.

91. The MYXV of claim 90, wherein the IL-12 α subunit and the IL-12 β subunit are joined by a polypeptide linker.

92. The MYXV of claim 91, wherein the polypeptide linker is an elastin linker.

93. The MYXV of claim 15, wherein the MYXV expresses a low level of the IL-12.

94. The MYXV of claim 15, wherein the MYXV expresses a high level of the IL-12.