

CLAIMS

1. A pharmaceutical composition comprising,
about 1×10^6 to about 1×10^{10} particle forming units (pfu)/ml of an oncolytic
5 vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide
encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a
functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion
in the SCR domains in the B5R membrane protein extracellular region; and
a pharmaceutically acceptable carrier.
10
2. The pharmaceutical composition of claim 1, wherein the pharmaceutically
acceptable carrier comprises tromethamine and sucrose.
3. The pharmaceutical composition of claim 2, wherein the pharmaceutically
15 acceptable carrier comprises tromethamine at a concentration of about 10 mmol/L to about 50
mmol/L.
4. The pharmaceutical composition of claim 2 or 3, wherein the pharmaceutically
acceptable carrier comprises sucrose at a concentration of about 5% w/v to about 15% w/v.
20
5. The pharmaceutical composition of any one of claims 1-4, wherein the pH of
the composition is about 5.0 to about 8.5.
6. A pharmaceutical composition comprising,
25 about 1×10^6 to about 1×10^{10} particle forming units (pfu)/ml of an oncolytic
vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide
encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a
functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion
in the SCR domains in the B5R membrane protein extracellular region;
30 tromethamine at a concentration of about 10 mmol/L to about 50 mmol/L; and
sucrose at a concentration of about 5% w/v to about 15% w/v,
wherein the pH of the composition is about 5.0 to about 8.5.
7. The pharmaceutical composition of any one of claims 1-6, wherein the
35 deletion in the SCR domains in the B5R membrane protein extracellular region comprises a
deletion in SCR domains 1-4.

8. The pharmaceutical composition of any one of claims 1-7, wherein the deletion in the SCR domains of the B5R region comprises amino acid residues 22-237 of the amino acid sequence set forth in GenBank Accession No. AAA48316.1.

5 9. The pharmaceutical composition of any one of claims 1-8, wherein the gene encoding the SCR domain-deleted B5R region is a gene encoding a polypeptide containing the signal peptide, stalk, transmembrane, and cytoplasmic tail domains of the B5R region.

10 10. The pharmaceutical composition according to any one of claims 1-9, wherein the SCR domain-deleted B5R region comprises the amino acid sequence of the B5R region corresponding to the amino acid sequence set forth in SEQ ID NO: 2.

11. The pharmaceutical composition of any one of claims 1-10, wherein the vaccinia virus is a LC16mo strain of virus.

15 12. The pharmaceutical composition of any one of claims 1-11, wherein the oncolytic vaccinia virus is LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7.

13. The pharmaceutical composition of any one of claims 1-12, comprising about 1×10^7 to about 1×10^9 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

20 14. The pharmaceutical composition of any one of claims 1-12, comprising about 1×10^7 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

25 15. The pharmaceutical composition of any one of claims 1-12, comprising about 5×10^7 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

16. The pharmaceutical composition of any one of claims 1-12, comprising about 1×10^8 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

30 17. The pharmaceutical composition of any one of claims 1-12, comprising about 5×10^8 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

18. The pharmaceutical composition of any one of claims 1-12, comprising about 1×10^9 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

35 19. The pharmaceutical composition of any one of claims 1-12, comprising about 5×10^9 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

20. The pharmaceutical composition of any one of claims 2-19, wherein the concentration of tromethamine is about 15 mmol/L to about 45 mmol/L; 20 mmol/L to about 40 mmol/L; or 25 mmol/L to about 35 mmol/L.

5 21. The pharmaceutical composition of claim 20, wherein the concentration of tromethamine is about 30 mmol/L.

22. The pharmaceutical composition of any one of claims 4-21, wherein the concentration of sucrose is about 6% w/v to about 14% w/v; about 7% w/v to about 13% w/v; 10 about 8% w/v to about 12% w/v; or about 9% w/v to about 11% w/v.

23. The pharmaceutical composition of claim 22, wherein the concentration of sucrose is about 10% w/v.

15 24. The pharmaceutical composition of any of claims 5-23, wherein the pH of the composition is about 6.0 to about 8.0; about 6.5 to about 8.0; or about 6.8 to about 7.8.

25. The pharmaceutical composition of claim 24, wherein the pH of the composition is about 7.6.

20

26. The pharmaceutical composition of any one of claims 1-25, wherein the composition is stable for at least about 6 months to about 2 years when stored at about -70°C.

27. A vial comprising the pharmaceutical composition of any one of claims 1-26.

25

28. A syringe comprising the pharmaceutical composition of any one of claims 1-26.

29. A method of treating a subject having a cancer, comprising administering to 30 the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1×10^6 to about 1×10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion 35 in the SCR domains in the B5R membrane protein extracellular region; and a pharmaceutically acceptable carrier, thereby treating the subject.

30. A method of treating a subject having a cancer, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1×10^6 to about 1×10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; and a pharmaceutically acceptable carrier, wherein administration of the pharmaceutical composition to the subject induces an abscopal effect, thereby treating the subject.
31. A method of inducing an abscopal effect in a subject having a cancer, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1×10^6 to about 1×10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; and a pharmaceutically acceptable carrier, thereby inducing an abscopal effect in a subject having a cancer.
32. A method of treating a subject having a cancer, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1×10^6 to about 1×10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; tromethamine at a concentration of about 10 mmol/L to about 50 mmol/L; and sucrose at a concentration of about 5% w/v to about 15% w/v, wherein the pH of the composition is about 5.0 to about 8.5, thereby treating the subject.
33. A method of treating a subject having a cancer, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1×10^6 to about 1×10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide

encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region;

- 5 tromethamine at a concentration of about 10 mmol/L to about 50 mmol/L; and
 sucrose at a concentration of about 5% w/v to about 15% w/v,
 wherein the pH of the composition is about 5.0 to about 8.5, and
 wherein administration of the pharmaceutical composition to the subject induces an
abscopal effect, thereby treating the subject.

- 10 34. A method of inducing an abscopal effect in a subject having a cancer,
 comprising administering to the subject a therapeutically effective amount of a
 pharmaceutical composition comprising,
 about 1×10^6 to about 1×10^{10} particle forming units (pfu)/ml of an oncolytic
 vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide
15 encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a
 functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion
 in the SCR domains in the B5R membrane protein extracellular region;
 tromethamine at a concentration of about 10 mmol/L to about 50 mmol/L; and
 sucrose at a concentration of about 5% w/v to about 15% w/v,
20 wherein the pH of the composition is about 5.0 to about 8.5, and
 wherein administration of the pharmaceutical composition to the subject induces an
abscopal effect, thereby inducing an abscopal effect in the subject.

- 25 35. The method of any one of claims 30, 31, 33, and 34, wherein the abscopal
effect occurs in a metastatic tumor that is proximate to a primary solid tumor.

36. The method of any one of claims 30, 31, 33, and 34, wherein the abscopal
effect occurs in a metastatic tumor that is remote to a primary solid tumor.

- 30 37. The method of any one of claims 29-36, wherein the oncolytic vaccinia virus
is LC16mO Δ SCR VGF-SP-IL12/O1L-SP-IL7.

38. The method of any one of claims 29-37, wherein the subject is administered a
dose of about 1×10^7 to about 1×10^9 particle forming units (pfu).

- 35 39. The method of any one of claims 29-37, wherein the subject is administered a
dose of about 1×10^7 particle forming units (pfu).

40. The method of any one of claims 29-37, wherein the subject is administered a dose of about 5×10^7 particle forming units (pfu).

5 41. The method of any one of claims 29-37, wherein the subject is administered a dose of about 1×10^8 particle forming units (pfu).

42. The method of any one of claims 29-37, wherein the subject is administered a dose of about 5×10^8 particle forming units (pfu).

10 43. The method of any one of claims 29-37, wherein the subject is administered a dose of about 1×10^9 particle forming units (pfu).

44. The method of any one of claims 29-37, wherein the administration is intratumoral administration.

15

45. The method of any one of claims 29-44, wherein the dose of the pharmaceutical composition is administered to the subject intratumorally in a volume that achieves an injection ratio of about 0.2 to about 0.8 (volume of pharmaceutical composition/tumor volume).

20

46. The method of any one of claims 29-44, wherein the pharmaceutical composition is administered to the subject once about once every week, once every two weeks, once every three weeks, or once every four weeks.

25

47. The method of any one of claims 29-44, wherein the pharmaceutical composition is administered to the subject once about once every two weeks.

48. The method of any one of claims 29-47, wherein the pharmaceutical composition is administered to the subject in a dosing regimen.

30

49. The method of claim 48, wherein the dosing regimen comprises administering to the subject a first dose of the pharmaceutical composition on day 1 and a second dose of the pharmaceutical composition on day 15.

35

50. The method of claim 49, wherein the dosing regimen is repeated beginning at day 28 following the first dose of the pharmaceutical composition.

51. The method of any one of claims 29-50, wherein the cancer is a primary tumor.

52. The method of claim 51, wherein the primary tumor is a solid tumor.
53. The method of claim 50, wherein the solid tumor is an advanced solid tumor.
- 5 54. The method of any one of claims 29-50, wherein the cancer is a metastatic tumor.
55. The method of any one of claims 29-50, wherein the cancer is a cutaneous, subcutaneous, mucosal or submucosal tumor.
- 10 56. The method of any one of claims 29-50, wherein the cancer is a primary or metastatic solid tumor in a location other than a cutaneous, a subcutaneous, a mucosal or a submucosal location.
- 15 57. The method of any one of claims 29-50, wherein the cancer is a head and neck squamous cell carcinoma, a dermatological cancer, a nasopharyngeal cancer, a sarcoma, or a genitourinary/gynecological tumor.
58. The method of any one of claims 29-50, wherein the cancer is a primary or
20 metastatic tumor of the liver.
59. The method of any one of claims 29-50, wherein the cancer is a primary or metastatic gastric tumor.
- 25 60. The method of any one of claims 29-50, wherein the cancer the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer
30 or gastric cancer.
61. The method of any one of claims 29-60, wherein the subject is human.
62. The method of any one of claims 29-61, wherein the subject is an adult subject.
- 35 63. The method of any one of claims 29-61, wherein the subject is an adolescent subject.

64. The method of any one of claims 29-61, wherein the subject is a pediatric subject.

65. The method of any one of claims 29-64, wherein administration of the pharmaceutical composition to the subject leads to at least one effect selected from the group consisting of inhibition of tumor growth, tumor regression, reduction in the size of a tumor, reduction in tumor cell number, delay in tumor growth, abscopal effect, inhibition of tumor metastasis, reduction in metastatic lesions over time, reduced use of chemotherapeutic or cytotoxic agents, reduction in tumor burden, increase in progression-free survival, increase in overall survival, complete response, partial response, antitumor immunity, and stable disease.

66. The method of any one of claims 29-65, further comprising administering to the subject an additional therapeutic agent or therapy,

67. The method of claim 66, wherein the additional therapeutic agent or therapy, is selected from the group consisting of surgery, radiation, a chemotherapeutic agent, a cancer vaccine, a checkpoint inhibitor, a lymphocyte activation gene 3 (LAG3) inhibitor, a glucocorticoid-induced tumor necrosis factor receptor (GITR) inhibitor, a T-cell immunoglobulin and mucin-domain containing-3 (TIM3) inhibitor, a B- and T-lymphocyte attenuator (BTLA) inhibitor, a T cell immunoreceptor with Ig and ITIM domains (TIGIT) inhibitor, a CD47 inhibitor, an indoleamine-2,3-dioxygenase (IDO) inhibitor, a bispecific anti-CD3/anti-CD20 antibody, a vascular endothelial growth factor (VEGF) antagonist, an angiopoietin-2 (Ang2) inhibitor, a transforming growth factor beta (TGF β) inhibitor, a CD38 inhibitor, an epidermal growth factor receptor (EGFR) inhibitor, granulocyte-macrophage colony stimulating factor (GM-CSF), cyclophosphamide, an antibody to a tumor-specific antigen, Bacillus Calmette-Guerin vaccine, a cytotoxin, an interleukin 6 receptor (IL-6R) inhibitor, an interleukin 4 receptor (IL-4R) inhibitor, an IL-10 inhibitor, IL-2, IL-7, IL-21, IL-15, an antibody-drug conjugate, an anti-inflammatory drug, and a dietary supplement.

68. The method of any one of claims 30-67, further comprising administering to the subject a therapeutically effective amount of a checkpoint inhibitor.

69. The method of claim 68, wherein the checkpoint inhibitor is a programmed cell death 1 (PD-1) inhibitor; a programmed cell death ligand 1 (PD-L1) inhibitor; a cytotoxic T lymphocyte associated protein 4 (CTLA-4) inhibitor; a T-cell immunoglobulin domain and mucin domain-3 (TIM-3) inhibitor; a lymphocyte activation gene 3 (LAG-3) inhibitor; a T cell immunoreceptor with Ig and ITIM domains (TIGIT) inhibitor; a B and T lymphocyte

associated (BTLA) inhibitor; or a V-type immunoglobulin domain-containing suppressor of T-cell activation (VISTA) inhibitor.

70. The method of claim 69, wherein the checkpoint inhibitor is a programmed cell death 1 (PD-1) inhibitor, a programmed cell death ligand 1 (PD-L1) inhibitor, or a cytotoxic T lymphocyte associated protein 4 (CTLA-4) inhibitor.

71. The methods of claim 69, wherein the checkpoint inhibitor is selected from the group consisting of an anti-PD-1 antibody, or antigen-binding fragment thereof; an anti-PD-L1 antibody, or antigen-binding fragment thereof; an anti-CTLA-4 antibody, or antigen-binding fragment thereof; an anti-TIM-3 antibody, or antigen-binding fragment thereof; an anti-LAG-3 antibody, or antigen-binding fragment thereof; an anti-TIGIT antibody, or antigen-binding fragment thereof; an anti-BTLA antibody, or antigen-binding fragment thereof; and an anti-VISTA antibody, or antigen-binding fragment thereof.

72. The method of claim 71, wherein the checkpoint inhibitor is an anti-programmed cell death 1 (PD-1) antibody, or antigen-binding fragment thereof; an anti-programmed cell death ligand 1 (PD-L1) antibody, or antigen-binding fragment thereof; or an anti-cytotoxic T lymphocyte associated protein 4 (CTLA-4) antibody, or antigen-binding fragment thereof.

73. The method of claim 72, wherein the anti-PD-1 antibody is nivolumab or pembrolizumab.

74. The method of claim 72, wherein the anti-PD-L1 antibody is atezolizumab.

75. The method of claim 72, wherein the anti-CTLA-4 antibody is ipilimumab.

76. A pharmaceutical composition comprising,
about 1×10^6 to about 1×10^{10} particle forming units (pfu)/ml of LC16mO Δ SCR VGF-SP-IL12/O1L-SP-IL7;
tromethamine at a concentration of about 30 mmol/L; and
sucrose at a concentration of about 10% w/v,
wherein the pH of the composition is about 7.6.

77. A method of treating a subject having a cancer, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising,

about 1×10^6 to about 1×10^{10} particle forming units (pfu)/ml of LC16mO
ΔSCR VGF-SP-IL12/O1L-SP-IL7;
tromethamine at a concentration of about 30 mmol/L; and
sucrose at a concentration of about 10% w/v,
5 wherein the pH of the composition is about 7.6, thereby treating the subject.

78. A method of treating a subject having a cancer, comprising administering to
the subject a therapeutically effective amount of a pharmaceutical composition comprising,
about 1×10^6 to about 1×10^{10} particle forming units (pfu)/ml of LC16mO
10 ΔSCR VGF-SP-IL12/O1L-SP-IL7;
tromethamine at a concentration of about 30 mmol/L; and
sucrose at a concentration of about 10% w/v,
wherein the pH of the composition is about 7.6, and
wherein administration of the pharmaceutical composition to the subject induces an
15 abscopal effect, thereby treating the subject.

79. A method of inducing an abscopal effect in a subject having a cancer,
comprising administering to the subject a therapeutically effective amount of a
pharmaceutical composition comprising,
20 about 1×10^6 to about 1×10^{10} particle forming units (pfu)/ml of LC16mO
ΔSCR VGF-SP-IL12/O1L-SP-IL7;
tromethamine at a concentration of about 30 mmol/L; and
sucrose at a concentration of about 10% w/v,
wherein the pH of the composition is about 7.6, and
25 wherein administration of the pharmaceutical composition to the subject induces an
abscopal effect, thereby inducing an abscopal effect in the subject.

30