

CLAIMS

We claim:

- 5 1. A recombinant oncolytic herpes simplex virus (HSV) comprising a nucleic acid construct encoding a fusion protein comprising an ScFv that specifically binds an immune checkpoint protein, wherein the ScFv is fused to a TGFβRII ectodomain (TGFβRII_{ecto}).
- 10 2. A recombinant oncolytic HSV according to claim 1, wherein the ScFv is derived from an anti-PD-1 monoclonal antibody or anti-PD-L1 monoclonal antibody.
- 15 3. A recombinant oncolytic HSV according to claim 2, wherein the ScFv is derived from an anti-PD-1 monoclonal antibody.
- 20 4. A recombinant oncolytic HSV according to claim 3, wherein the anti-PD-1 ScFv comprises a heavy chain variable region sequence having at least 95% identity to SEQ ID NO:8 and a light chain variable region sequence having at least 95% identity to SEQ ID NO:9.
- 25 5. A recombinant oncolytic HSV according to claim 4, wherein the anti-PD-1 ScFv has at least 95% identity to SEQ ID NO:11.
- 30 6. A recombinant oncolytic HSV according to claim 3, wherein the anti-PD-1 ScFv comprises a heavy chain variable region sequence having at least 95% identity to SEQ ID NO:12 and a light chain variable region sequence having at least 95% identity to SEQ ID NO:13.
- 35 7. A recombinant oncolytic HSV according to claim 6, wherein the anti-PD-1 ScFv has at least 95% identity to SEQ ID NO:15.
- 40 8. A recombinant oncolytic HSV according to claim 3, wherein the anti-PD-1 ScFv comprises a heavy chain variable region sequence having at least 95% identity to SEQ ID NO:16 and a light chain variable region sequence having at least 95% identity to SEQ ID NO:17.
- 45 9. A recombinant oncolytic HSV according to claim 8, wherein the anti-PD-1 ScFv has at least 95% identity to SEQ ID NO:19.
10. A recombinant oncolytic HSV according to claim 2, wherein the ScFv is derived from an anti-PD-L1 monoclonal antibody.
11. A recombinant oncolytic HSV according to claim 10, wherein the anti-PD-L1 ScFv comprises a heavy chain variable region sequence having at least 95% identity to SEQ ID NO:20 and a light chain variable region sequence having at least 95% identity to SEQ ID NO:21.

12. A recombinant oncolytic HSV according to claim 11, wherein the anti-PD-L1 ScFv has at least 95% identity to SEQ ID NO:23.
- 5 13. A recombinant oncolytic HSV according to claim 10, wherein the anti-PD-L1 ScFv comprises a heavy chain variable region sequence having at least 95% identity to SEQ ID NO:24 and a light chain variable region sequence having at least 95% identity to SEQ ID NO:25.
- 10 14. A recombinant oncolytic HSV according to claim 13, wherein the anti-PD-L1 ScFv has at least 95% identity to SEQ ID NO:27.
- 15 15. A recombinant oncolytic HSV according to claim 10, wherein the anti-PD-1 ScFv comprising a heavy chain variable region sequence having at least 95% identity to SEQ ID NO:28 and a light chain variable region sequence having at least 95% identity to SEQ ID NO:29.
16. A recombinant oncolytic HSV according to claim 15, wherein the anti-PD-L1 ScFv has at least 95% identity to SEQ ID NO:31.
- 20 17. A recombinant oncolytic HSV according to claim 1, wherein the ScFv is fused to TGFβRII_{ecto} via an Fc region.
- 25 18. A recombinant oncolytic HSV according to claim 1, wherein the nucleic acid construct comprises a promoter operable in a mammalian cell operably linked to the fusion protein-encoding sequence.
- 30 19. A recombinant oncolytic HSV according to claim 18, wherein the promoter is selected from the group consisting of EF1α/HTLV, CMV, and Jet.
- 35 20. A recombinant oncolytic HSV according to any of the previous claims, wherein the nucleic acid construct comprises a sequence encoding a signal peptide 5' of the sequence encoding the ScFv.
- 40 21. A recombinant oncolytic HSV according to any of the previous claims, wherein TGFβRII ectodomain comprises an amino acid sequence having at least 95% identity to SEQ ID NO:7.
22. A recombinant oncolytic HSV according to claim 21, wherein TGFβRII ectodomain comprises SEQ ID NO:7.
23. A recombinant oncolytic HSV according to claim 17, wherein the Fc region is an IgG1 Fc region or an IgG4 Fc region.
- 45 24. A recombinant oncolytic HSV according to claim 23, wherein the Fc region has at least 95% identity to SEQ ID NO:5.

25. A recombinant oncolytic HSV according to claim 24, wherein the Fc region comprises SEQ ID NO:5.
- 5 26. A recombinant oncolytic HSV according to claim 23, wherein the Fc region has at least 95% identity to SEQ ID NO:2.
27. A recombinant oncolytic HSV according to claim 26, wherein the Fc region comprises SEQ ID NO:2.
- 10 28. A recombinant oncolytic HSV according to any of claims 1-27, further comprising a gene encoding IL12.
29. A recombinant oncolytic HSV according to claim 28, wherein the gene encoding IL12 encodes a polypeptide having at least 90% identity to human IL12 (SEQ ID NO:52).
- 15 30. A recombinant oncolytic HSV according to claim 28, wherein the gene encoding IL12 encodes a polypeptide having at least 90% identity to murine IL12 (SEQ ID NO:54).
31. A recombinant oncolytic HSV according to any of claims 28-30, wherein the IL12 gene is operably linked to a second promoter operable in a mammalian cell.
- 20 32. A recombinant oncolytic HSV according to claim 31, wherein the promoter is selected from the group consisting of EF1 α /HTLV, CMV, and Jet.
- 25 33. A recombinant oncolytic HSV comprising a nucleic acid construct encoding a fusion protein comprising an anti-PD-1 ScFv (SEQ ID NO:11) linked to TGF β RII_{ecto} (SEQ ID NO:7) via an Fc4 region (SEQ ID NO:2).
- 30 34. A recombinant oncolytic HSV according to claim 33, wherein the fusion protein comprises SEQ ID NO:40.
- 35 35. A recombinant oncolytic HSV comprising a nucleic acid construct encoding a fusion protein comprising an anti-PD-1 ScFv (SEQ ID NO:15) linked to TGF β RII_{ecto} (SEQ ID NO:7) via an Fc4 region (SEQ ID NO:2).
36. A recombinant oncolytic HSV according to claim 37, wherein the fusion protein comprises SEQ ID NO:42.
- 40 37. A recombinant oncolytic HSV comprising a nucleic acid construct encoding a fusion protein comprising an anti-PD-1 ScFv (SEQ ID NO:19) linked to TGF β RII_{ecto} (SEQ ID NO:7) via the Fc4 region (SEQ ID NO:2).
- 45 38. A recombinant oncolytic HSV according to claim 41, wherein the fusion protein comprises (SEQ ID NO:44).

39. A recombinant oncolytic HSV comprising a nucleic acid construct encoding a fusion protein comprising an anti-PD-1 ScFv (SEQ ID NO:23) linked to TGFβRII_{ecto} (SEQ ID NO:7) via an Fc4 region (SEQ ID NO:2).
- 5 40. A recombinant oncolytic HSV according to claim 33, wherein the fusion protein comprises SEQ ID NO:46.
- 10 41. A recombinant oncolytic HSV comprising a nucleic acid construct encoding a fusion protein comprising an anti-PD-1 ScFv (SEQ ID NO:27) linked to TGFβRII_{ecto} (SEQ ID NO:7) via an Fc4 region (SEQ ID NO:2).
42. A recombinant oncolytic HSV according to claim 37, wherein the fusion protein comprises SEQ ID NO:48.
- 15 43. A recombinant oncolytic HSV comprising a nucleic acid construct encoding a fusion protein comprising an anti-PD-1 ScFv (SEQ ID NO:31) linked to TGFβRII_{ecto} (SEQ ID NO:7) via the Fc4 region (SEQ ID NO:2).
- 20 44. A recombinant oncolytic HSV according to claim 41, wherein the fusion protein comprises (SEQ ID NO:50).
45. A recombinant oncolytic HSV according to any of claims 33-44, further comprising a gene encoding human IL12.
- 25 46. A recombinant oncolytic HSV according to claim 45, wherein the gene encoding human IL12 encodes the polypeptide of SEQ ID NO:52 or a polypeptide having at least 95% identity thereto.
- 30 47. A recombinant oncolytic HSV according to any of the previous claims, wherein the oncolytic HSV is an HSV-1.
48. A recombinant oncolytic HSV according to claim 33, wherein the oncolytic HSV is derived from HSV-1 strain 17, HSV-1 strain F, HSV-1 strain KOS, or HSV-1 strain JS1.
- 35 49. A recombinant oncolytic HSV according to claim 34, wherein the oncolytic HSV is derived from HSV strain 17.
50. A recombinant oncolytic HSV according to any of the previous claims, wherein the oncolytic HSV does not encode a functional ICP34.5-encoding gene.
- 40 51. A recombinant oncolytic HSV according to claim 36, wherein all or a portion of the ICP34.5-encoding gene is deleted.
- 45 52. A recombinant oncolytic HSV according to claim 36 or 37, wherein the nucleic acid construct encoding the fusion protein and/or the gene encoding IL12 are inserted into the ICP34.5-encoding gene locus.

53. A recombinant oncolytic HSV for use in a method of treating cancer, wherein the method comprises administering an oncolytic HSV according to any of claims 1-38 to a subject having cancer.
- 5 54. A recombinant oncolytic HSV according to claim 53, for use in a method comprising administering the oncolytic HSV by intravenous, intracavitary, intraperitoneal, intratumoral, or peritumoral delivery.
- 10 55. A recombinant oncolytic HSV according to claim 53 or 54, wherein the method comprises administering more than one dose of the oncolytic HSV to the patient.
56. A recombinant oncolytic HSV according to any of claims 53-55, wherein the cancer is a solid tumor.
- 15 57. A recombinant oncolytic HSV according to any of claims 53-56, wherein the subject is a human.
58. A recombinant oncolytic HSV according to any of claims 53-56, wherein the subject is a dog.
- 20 59. A pharmaceutical composition comprising a recombinant oncolytic HSV according to any of claims 1-58.
60. A pharmaceutical composition according to claim 59, wherein the oncolytic HSV is at a concentration of at least 10^6 per ml.
- 25 61. A pharmaceutical composition according to claim 60, wherein the oncolytic HSV is at a concentration of at least 10^7 per ml.
- 30 62. A method of treating cancer in a subject, comprising administering an oncolytic HSV or pharmaceutical composition according to any of claims 1-61 to a subject having cancer.
63. A method according to claim 62, wherein the subject is a human.
- 35 64. A method according to claim 62, wherein the subject is a dog.
65. A method according to claim 63 or 64, comprising administering the oncolytic HSV by intravenous, intra-arterial, intracavitary, intratumoral, or peritumoral delivery.
- 40 66. A method according to claim 65, comprising administering more than one dose of the oncolytic HSV to the subject.
67. A method according to any of claims 60-66, wherein the cancer is a solid tumor.
- 45 68. A fusion protein comprising a single chain variable fragment (ScFv) that binds an immune checkpoint protein, a TGF β RII ectodomain (TGF β RII_{ecto}), and an Fc antibody region linking the ScFv to the TGF β RII_{ecto}.

69. A fusion protein according to claim 68, wherein the immune checkpoint protein is PD-1 or PD-L1.
70. A fusion protein according to claim 69, wherein the immune checkpoint protein is PD-1.
71. A fusion protein according to claim 70, wherein the ScFv is derived from a BB9 anti-PD-1 monoclonal antibody, an RG1H10 anti-PD-1 monoclonal antibody, or pembrolizumab.
72. A fusion protein according to claim 71, wherein the ScFv comprises a sequence having at least 95% identity to SEQ ID NO:11, SEQ ID NO:15, or SEQ ID NO:19.
73. A fusion protein according to claim 69, wherein the immune checkpoint protein is PD-L1.
74. A fusion protein according to claim 73, wherein the ScFv is derived from a Combi5 anti-PD-L1 monoclonal antibody, an H6B1LEM anti-PD-L1 monoclonal antibody, or avelumab.
75. A fusion protein according to claim 74, wherein the ScFv comprises a sequence having at least 95% identity to SEQ ID NO:23, SEQ ID NO:27, or SEQ ID NO:31.
76. A fusion protein according to any of claims 68-75, wherein the TGF β RII_{ecto} comprises an amino acid sequence having at least 95% identity to SEQ ID NO:7.
77. A fusion protein according to claim 76, wherein the TGF β RII_{ecto} comprises SEQ ID NO:7.
78. A fusion protein according to any of claims 68-77, wherein the Fc is an IgG1 Fc or an IgG4 Fc.
79. A fusion protein according to claim 68, wherein the Fc is a human Fc.
80. A fusion protein according to claim 79, wherein the Fc comprises an amino acid sequence having at least 95% identity to SEQ ID NO:2 or SEQ ID NO:5.
81. A conditioned media composition comprising a fusion protein according to any of claims 68-80.
82. A conditioned media composition according to claim 81, wherein the cell supernatant is virus-free.
83. A pharmaceutical composition comprising a fusion protein according to any of claims 68-80.
84. A method of treating cancer, comprising administering a pharmaceutical composition according to claim 83 to a subject having cancer.
85. A method according to claim 84, wherein the subject is a human.

86. A method according to claim 84, wherein the subject is a dog.
87. A nucleic acid construct comprising a nucleic acid sequence encoding a fusion protein according to any of claims 68-80.
- 5 88. A nucleic acid construct according to claim 87, wherein the nucleic acid sequence encoding a fusion protein is operably linked to a promoter.
- 10 89. A nucleic acid construct according to claim 88, wherein the promoter is a eukaryotic promoter.
90. A nucleic acid construct according to claim 89, wherein the promoter is operable in a mammalian cell.
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