

CLAIMS

What is claimed is:

1. 1. A fusion protein comprising:
 - ~~a. an antibody or a fragment of said antibody or a variant of said antibody that binds to programmed cell death protein-1 (PD-1); and~~
 - ~~a Transforming growth factor beta (TGF- β) cytokine trap; (a) an IgG4 antibody comprising:~~
 - ~~(i) a first polypeptide comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 6 or 7; (ii) a second polypeptide comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 12 or 13; and (iii) a third polypeptide comprising the amino acid sequence of SEQ ID NO: 146 but with a mutation at position 108 thereof; and~~
 - ~~(b) a fourth polypeptide comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 14;~~
 - ~~b. wherein one or more polypeptides of the fusion protein are connected by a linker.~~
- ~~2. The fusion protein of claim 1, wherein said linker comprises (G4S)_n, wherein n is 2, the amino acid sequence of any one of SEQ ID NOs: 17-34.~~
 - ~~2. 3, 4, 5, or 6.~~
 - ~~3. The fusion protein of claim 1, wherein said linker comprises (Gly)_n, wherein n is 6, 7, or 8.~~
 - ~~4. The fusion protein of claim 1, wherein said linker the third polypeptide comprises an amino acid sequence that differs from the amino acid sequence of SEQ ID NO: 14 only by conservative amino acid substitutions.~~
 - ~~4. The fusion protein of claim 1, wherein the mutation is a S108P mutation.~~
 5. The fusion protein of claim 1, wherein the linker connects the first polypeptide to the fourth polypeptide.

4. ~~6.~~ The fusion protein of claim 1, wherein the first polypeptide comprises ~~(EAAAK)_n~~, wherein ~~n~~ is 1, 2, 3, 4, 5, or 6, an amino acid sequence that differs from the amino acid sequence of SEQ ID NO: 6 or 7 only by conservative amino acid substitutions.

5. ~~7.~~ The fusion protein of claim 1, wherein ~~said linker~~ the second polypeptide comprises ~~A(EAAAK)₄LEA(EAAAK)₄A~~ an amino acid sequence that differs from the amino acid sequence of SEQ ID NO: 12 or 13 only by conservative amino acid substitutions.

8. ~~A pharmaceutical composition comprising:~~
~~the fusion protein of claim 1; and~~
~~a pharmaceutically acceptable excipient.~~

6. ~~9.~~ The fusion protein of claim 1, ~~wherein said linker comprises a~~ comprising: a polypeptide having at least 95% sequence identity with SEQ ID NOs: 17-34.

7. ~~The fusion protein of any one of claims 1 to 6, wherein said TGF- β cytokine trap comprises transforming growth factor receptor (TGF β R), or a functional fragment thereof, an anti-TGF- β antibody or an antigen binding fragment thereof, NO: 296 and a TGF- β 1 inhibitory peptide or a variant thereof~~ polypeptide having at least 95% sequence identity with SEQ ID NO: 295.

8. ~~10.~~ The fusion protein of claim 7, ~~wherein said TGF β R is transforming growth factor beta receptor II (TGF β RII) or said functional fragment thereof.~~

9. ~~The fusion protein of any one of claim 8, wherein said functional fragment of TGF β RII is~~ 1, comprising: a polypeptide having at least 95% sequence identity with SEQ ID NO: 15 and a TGF β RII extracellular domain (ECD); polypeptide having at least 95% sequence identity with SEQ ID NO: 294.

10. ~~The fusion protein of claim 9, wherein said ECD binds TGF- β 1.~~

11. ~~The fusion protein of claim 9, wherein said ECD binds TGF- β 3.~~

~~12.~~ 11. The fusion protein of claim ~~9~~, wherein said ECD binds TGF- β 1 and TGF- β 31, comprising: the amino acid sequence of SEQ ID NO: 15; and the amino acid sequence of SEQ ID NO: 294.

~~13.~~ 12. The fusion protein of claim ~~12~~, wherein said ECD binds TGF- β 1 and TGF- β 3 but not TGF- β 2.1, comprising: the amino acid sequence of SEQ ID NO: 296; and the amino acid sequence of SEQ ID NO: 295.

The fusion protein of any one of claims 1 to

~~14.~~ 13. The fusion protein of claim ~~1~~, wherein said TGF- β cytokine trap the first polypeptide comprises a the amino acid sequence at least 80% identical to a sequence as shown in of SEQ ID NO: 14, SEQ ID NO. 141, ~~6~~ or SEQ ID NO: 142~~7~~.

~~15.~~ The fusion protein of any one of claims 1 to ~~13~~, wherein said TGF- β cytokine trap comprises a sequence as shown in SEQ ID NO: 14, SEQ ID NO. 141, or SEQ ID NO: 142.

~~16.~~ The fusion protein of any one of claims 1 to ~~15~~, wherein said TGF- β cytokine trap comprises a sequence as shown in SEQ ID NO: 14.

~~17.~~ The fusion protein of any one of claims 1 to ~~16~~, wherein said antibody (anti PD1) is immunoglobulin G (IgG) antibody.

~~18.~~ The fusion protein of claim ~~17~~, wherein said IgG is IgG1, IgG2, IgG3, or IgG4.

~~19.~~ 14. The fusion protein of claim ~~18~~¹, wherein said IgG~~4~~ the second polypeptide comprises a mutation at position ~~108~~ the amino acid sequence of SEQ ID NO ~~146~~: 12 or SEQ ID NO: 292, 13.

~~20.~~ 15. The fusion protein of claim ~~19~~¹, wherein said mutation is S108P mutation.

~~21.~~ The fusion protein of any one of claims 18 to ~~20~~, wherein said IgG4 is connected to said TGF- β cytokine trap by said linker.

22. — The fusion protein of any one of claims 1 to 21, wherein said fragment of said antibody is a Fab, (Fab)₂, (Fab')₂, Fv, (Fv)₂, or scFv.

23. — The fusion protein of any one of claims 1 to 22, wherein said antibody the fourth polypeptide comprises a variable region of heavy chain (V_H) and a variable region of light chain (V_L) of said antibody.

24. — The fusion protein of claim 23, wherein said linker connects said variable region of heavy chain (V_H) to said TGF-β cytokine trap.

25. — The fusion protein of claim 23, wherein said linker connects said variable region of light chain (V_L) to said TGF-β cytokine trap.

26. — The fusion protein of any one of claims 23 to 25, wherein said variable region of heavy chain (V_H) is connected to said variable region of light chain (V_L) by a second linker in said fusion protein.

27. — The fusion protein of claim 26, wherein said second linker comprises a sequence as shown in any one of SEQ ID NOs: 17-34.

28. — The fusion protein of any one of claims 23 to 27, wherein said variable region of heavy chain (V_H) is at least 80% identical to a sequence as shown in any one of SEQ ID NOs: 1-7 and 149-164.

29. — The fusion protein of any one of claims 23 to 28, wherein said variable region of light chain (V_L) is at least 80% identical to a sequence as shown in any one of SEQ ID NOs: 8-13 and 148.

30. — The fusion protein of any one of claims 23 to 29, wherein said variable region of heavy chain (V_H) comprises a sequence as shown in any one of SEQ ID NOs: 1-7 and 149-164

31. — The fusion protein of any one of claims 23 to 30, wherein said variable region of light chain (V_L) comprises a sequence as shown in any one of SEQ ID NOs: 8-13 and 148.

32. — The fusion protein of any one of claims 23 to 29, wherein said variable region of heavy chain (V_H) is at least 90% identical to a sequence as shown in SEQ ID NO: 6 and said variable region of light chain (V_L) is at least 90% identical to a sequence as shown in SEQ ID NO: 12.

33. — The fusion protein of claim 32, wherein said variable region of heavy chain (V_H) comprises a sequence as shown in SEQ ID NO: 6 and said variable region of light chain (V_L) comprises a sequence as shown in SEQ ID NO: 12.

34. — The fusion protein of any one of claims 1 to 33, wherein said fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 16.

35. — The fusion protein of any one of claims 1 to 33, wherein said fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 143.

36. — The fusion protein of any one of claims 23 to 29, wherein said variable region of heavy chain (V_H) is at least 90% identical to a sequence as shown in SEQ ID NO: 7 and said variable region of light chain is at least 90% identical to a sequence as shown in SEQ ID NO: 13.

37. — The fusion protein of claim 36, wherein said variable region of heavy chain (V_H) comprises a sequence as shown in SEQ ID NO: 7 and said variable region of light chain (V_L) comprises the amino acid sequence as shown in SEQ ID NO: 13.

38. — The fusion protein of any one of claims 1 31 and claims 36 37, wherein said fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 145.

39. — The fusion protein of any one of claims 1 31 and claims 36 37 wherein said fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 144.

40. — The fusion protein of any one of claims 1 to 39, wherein said antibody further comprises fragment crystallizable region (F_C).

41. ~~— The fusion protein of claims 40, wherein said F_C is human F_C1, F_C2, F_C3, F_C4, or a fragment thereof.~~
42. ~~— The fusion protein of claims 40, wherein said F_C further comprises one or more mutations.~~
43. ~~The fusion protein of any one of claims 22 to 42, wherein said antibody comprises said scFv and said F_C fragment.~~
44. ~~The fusion protein of any one of claims 7 to 43, wherein said TGF β cytokine trap comprises said anti-TGF β antibody or said antigen binding fragment thereof.~~
45. ~~The fusion protein of claim 44, wherein the anti-TGF β antibody comprises VH encoded by SEQ ID NOs: 166, 168, 169, 171, 173, 175, or 177, and VL encoded by SEQ ID NOs: 165, 167, 170, 172, 174, 176, or 178.~~
46. ~~The fusion protein of any one of claims 7 to 43, wherein said TGF β cytokine trap comprises said TGF β inhibitory peptides or said variant thereof.~~
47. ~~The fusion protein of claim 46, wherein the TGF β inhibitory peptides comprises sequence set forth in any one of SEQ ID NOs. 193-227.~~
48. ~~A polynucleotide encoding the fusion protein of any one of claims 1 to 47 and 117 to 120~~
49. ~~An expression vector comprising a polynucleotide encoding the fusion protein of any one of claims 1 to 47 and 117 to 120, wherein said polynucleotide is operably linked to a promoter.~~
50. ~~The expression vector of claim 49, wherein said promoter is a constitutive promoter, a tissue specific promoter, or an inducible promoter.~~
51. ~~The expression vector of claim 50, wherein said inducible promoter is a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch.~~
52. ~~The expression vector of any one of claims 49 to 51, wherein said vector is an adenoviral vector.~~

53. A pharmaceutical composition, comprising:

- (a) the fusion protein of any one of claims 1 to 47 and 117 to 120;
- (b) a polynucleotide encoding the fusion protein of any one of claims 1 to 47 and 117 to 120; or
- (c) the expression vector of any one of claims 49 to 52; and
- (d) ~~of a pharmaceutically acceptable excipient.~~

54. A method of treating cancer, comprising: contacting a cell with

- (a) the fusion protein of any one of claims 1 to 47 and 117 to 120;
- (b) a polynucleotide encoding the fusion protein of any one of claims 1 to 47 and 117 to 120; or
- (c) the expression vector of any one of claims 49 to 52.

55. The method of claim 54, wherein said cell is a cancer cell.

56. The method of any one of claims 54 to 55, wherein said cell is a mammalian cell.

57. A method of treating a subject with a cancer, the method comprising: administering a composition comprising a fusion protein comprising

- (a) an antibody or a fragment or a variant of said antibody that binds to programmed cell death protein 1 (PD-1); and
- (b) a transforming growth factor receptor (TGF β R) or a functional fragment thereof, an anti TGF β antibody or an antigen binding fragment thereof, a TGF β 1 inhibitory peptide or a variant thereof;

wherein one or more polypeptides of the fusion protein are connected by a linker.

58. The method of claim 57, wherein said linker comprises (G4S)_n, wherein n is 2, 3, 4, 5, or 6.

59. The method of claim 57, wherein said linker comprises (Gly)_n, wherein n is 6, 7, or 8.

60. The method of claim 57, wherein said linker comprises (EAAAK)_n, wherein n is 1, 2, 3, 4, 5, or 6.

61. The method of claim 57, wherein said linker comprises A(EAAAK)₄LEA(EAAAK)₄A.

62. The method of claim 57, wherein said linker comprises a sequence as shown in any one of SEQ ID NOs: 17-34.

63. The method of any one of claims 57 to 62, wherein said transforming growth factor receptor protein is TGF β RII.

64. The method of claim 63, wherein said functional fragment of said TGF β RII is TGF β RII extracellular domain (ECD).

65. The method of any one of claims 57 to 64, wherein said TGF β cytokine trap comprises a sequence at least 80% identical to a sequence as shown in SEQ ID NO: 14, SEQ ID NO: 141, or SEQ ID NO: 142.

66. The method of any one of claims 57 to 64, wherein said TGF β cytokine trap comprises a sequence as shown in SEQ ID NO: 14, SEQ ID NO: 141, or SEQ ID NO: 142.

67. The method of any one of claims 57 to 66, wherein said antibody is immunoglobulin G (IgG) antibody.

68. The method of any one of claims 57 to 67, wherein said IgG is IgG1, IgG2, IgG3, or IgG4.

69. The method of claim 68, wherein said IgG4 comprises a mutation at position 108 of SEQ ID NO: 146 or SEQ ID NO: 292.

70. The method of claim 69, wherein said mutation is S108P mutation.

71. The method of claim 57, wherein said fragment of said antibody is a Fab, (Fab)₂, (Fab')₂, Fv, (Fv)₂, or scFv of said antibody.

72. The method of any one of claims 57 to 71, wherein said antibody or a fragment of said antibody or a variant of said antibody comprises a variable region of heavy chain (V_H) and a variable region of light chain (V_L).

73. The method of claim 72, wherein said linker connects said variable region of heavy chain (V_H) to said TGF β cytokine trap.

74. The method of claim 72, wherein said linker connects said variable region of light chain (V_L) and to said TGF- β cytokine trap.
75. The method of any one of claims 72 to 74, wherein said variable region of heavy chain (V_H) is connected to said variable region of light chain (V_L) by a second linker.
76. The method of any one of claims 72 to 75, wherein said variable region of heavy chain (V_H) is at least 80% identical to a sequence as shown in any one of SEQ ID NOs: 1-7 and 149-164.
77. The method of any one of claims 72 to 76, wherein said variable region of light chain (V_L) is at least 80% identical to a sequence as shown in any one of SEQ ID NOs: 8-13 and 148.
78. The method of any one of claims 72 to 77, wherein said variable region of heavy chain (V_H) comprises a sequence as shown in any one of SEQ ID NOs: 1-7 and 149-164
79. The method of any one of claims 72 to 78, wherein said variable region of light chain (V_L) comprises a sequence as shown in any one of SEQ ID NOs: 8-13 and 148.
80. The method of any one of claims 72 to 77, wherein said variable region of heavy chain (V_H) is at least 90% identical to a sequence as shown in SEQ ID NO: 6 and said variable region of light chain (V_L) is at least 90% identical to a sequence as shown in SEQ ID NO: 12.
81. The method of claim 80, wherein said variable region of heavy chain (V_H) comprises a sequence as shown in SEQ ID NO: 6 and said variable region of light chain (V_L) comprises a sequence as shown in SEQ ID NO: 12.
82. The method of any one of claims 57 to 81, wherein said fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 16.
83. The method of any one of claims 57 to 81, wherein said fusion protein comprises a sequence as shown in SEQ ID NO: 15 (VL5 IgG4) and a sequence as shown in SEQ ID NO: 143.

84. The method of any one of claims 72 to 77, wherein said variable region of heavy chain (VH) is at least 90% identical to a sequence as shown in SEQ ID NO: 7 and said variable region of light chain (VL) is at least 90% identical to a sequence as shown in SEQ ID NO: 13.
85. The method of claim 84, wherein said variable region of heavy chain comprises a sequence as shown in SEQ ID NO: 7 and said variable region of light chain comprises a sequence as shown in SEQ ID NO: 13.
86. The method of any one of claims 57 to 79 and claims 84 to 85, wherein said fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 145.
87. The method of any one of claims 57 to 79 and claims 84 to 85, wherein said fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 144.
88. The method of any one of claims 57 to 87, wherein said antibody further comprises fragment crystallizable region (Fc).
89. The method of claims 88, wherein said Fc is human Fc1, Fc2, Fc3, Fc4, or a fragment thereof.
90. The method of claims 88, wherein said Fc further comprises one or more mutations.
91. The method of any one of claims 71 to 90, wherein said antibody comprises said scFv and said Fc fragment.
92. The method of any one of claims 57 to 91, wherein said cancer is a refractory cancer.
93. The method of any one of claims 57 to 92, wherein said subject is non-responsive to a treatment with a PD-1 antibody or a CTLA-4 antibody.
94. The method of any one of claims 57 to 93, further comprising administering one or more additional anti-cancer agent.

95. ~~The method of claim 94, wherein said additional anti-cancer agent is a PD-1 inhibitor, PD-L1 inhibitor or a CTLA-4 inhibitor.~~
96. ~~The method of claim 95, wherein said PD-1 inhibitor is an anti-PD-1 antibody or a fragment or a variant thereof.~~
97. ~~The method of claim 95, wherein said CTLA-4 inhibitor is an anti-CTLA-4 antibody or a fragment or a variant thereof.~~
98. ~~The method of any one of claims 57 to 97, further comprising administering one or more cytokines.~~
99. ~~The method of any one of claims 57 to 98, wherein said subject is a mammalian subject.~~
100. ~~— The method of any one of claims 57 to 99, wherein said subject is a human.~~
101. ~~— The method of any one of claims 57-100, wherein said cancer is mesothelioma, glioblastoma, endometrial cancer, colorectal cancer, gastric cancer, cervical cancer, ovarian cancer, pancreatic cancer, prostate cancer, breast cancer, stomach cancer, bladder cancer, liver cancer, Hodgkin's lymphoma, lung cancer, skin cancer, renal cancer or head and neck cancer.~~
102. ~~— The method of claim 101, wherein skin cancer is cutaneous squamous cell carcinoma, melanoma or basal cell cancer.~~
103. ~~— The method of claim 101, where lung cancer is non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC).~~
104. ~~— The method of claim 101, wherein breast cancer is triple negative breast cancer (TNBC).~~
105. ~~— The method of any one of claims 57 to 104, further comprising administering an effective amount of T cells engineered to express an exogenous receptor.~~
106. ~~— The method of claim 105, wherein the exogenous receptor is a chimeric antigen receptor.~~

- 107.— The method of claim 106, wherein the chimeric antigen receptor is an engineered T-cell receptor.
- 108.— The method of any one of claims 106 to 107, wherein the chimeric antigen receptor comprises an antigen binding domain that binds to an epitope on CD19, BCMA, CD44, α -Folate receptor, CAIX, CD30, ROR1, CEA, EGP-2, EGP-40, HER2, HER3, Folate binding Protein, GD2, GD3, IL-13R α 2, KDR, EDB-F, mesothelin, CD22, EGFR, Folate receptor α , MUC-1, MUC-4, MUC-16, MAGE-A1, h5T4, PSMA, TAG-72, EGFR, CD20, EGFRvIII, CD123 or VEGF-R2.
- 109.— The method of claim 108, wherein the antigen binding domain comprises a sequence selected from SEQ ID NOs: 37-56.
- 110.— The method of claim 108, wherein the antigen binding domain comprises a sequence selected from SEQ ID NOs: 35-36.
- 111.— The method of any one of claims 107-110, wherein an effective amount of engineered T-cells is at least 10^2 -cells/kg.
- 112.— The method of any one of claims 107-111, wherein an effective amount of engineered T-cells is at least 10^4 -cells/kg.
- 113.— The method of any one of claims 107-112, wherein an effective amount of engineered T-cells is at least 10^5 -cells/kg.
- 114.— The method of any one of claims 107-113, wherein the engineered T-cells further express a cytokine.
- 115.— The method of claim 114, wherein the cytokine is a fusion protein comprising IL-15 and IL-15R α .
- 116.— A method of treating cancer in a subject in need thereof comprising
- (a) administering a composition comprising a fusion protein comprising an antibody or a fragment of said antibody or a variant of said antibody that binds to programmed cell death

protein 1 (PD-1); and a transforming growth factor receptor (TGF β R) protein or a functional fragment thereof; wherein one or more polypeptides of the fusion protein are connected by a linker; and

(b) administering to the subject one or more doses of an effective amount of engineered T-cells, wherein the engineered T-cells comprise a chimeric receptor and membrane bound IL-15.

117. — The fusion protein of any one of claims 1 to 33, wherein said fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 294.

118. — The fusion protein of any one of claims 1 31 and claims 36 37 wherein said fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 295.

119. — The method of any one of claims 57 to 81, wherein said fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 294.

120. — The method of any one of claims 57 to 79 and claims 84 to 85, wherein said fusion protein comprises a sequence as shown in SEQ ID NO: 13 and a sequence as shown in SEQ ID NO: 295.