

FUSION CONSTRUCTS AND METHODS OF USING THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Patent Application Nos. 62/695,623 filed on July 9, 2018, 62/695,627 filed on July 9, 2018, 62/863,710 filed June 19, 2019, 62/864,367 filed June 20, 2019 and 62/866,420 filed June 25, 2019, which are hereby incorporated by reference in their entireties.

BACKGROUND OF THE DISCLOSURE

[0002] Recently, monoclonal antibody-based cancer immunotherapy based on the interruption of suppressive signals that are delivered to the adaptive immune system has shown promise in the clinic. With the FDA approval of CTLA-4 antibody inhibitor (*e.g.*, ipilimumab) and PD-1 inhibitors (*e.g.*, pembrolizumab, nivolumab), more treatment options are now available to treat solid tumors including lung cancer, renal cell cancer, and ovarian cancer. However, in the majority of indications where PD-1/PD-L1 and TGF- β are co-expressed (*e.g.*, ovarian, gastric and colorectal) little to no response to immune checkpoint inhibitors has been observed. Accordingly, there is a continuing need in the art to obtain safer and more effective treatments for cancer.

INCORPORATION BY REFERENCE

[0003] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

SUMMARY OF THE DISCLOSURE

[0004] Provided herein is 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 (b) a Transforming growth factor beta (TGF- β) cytokine trap; wherein one or more polypeptides of the fusion protein are connected by a linker.

[0005] In one embodiment, said linker comprises (G4S) $_n$, wherein n is 2, 3, 4, 5, or 6. In one embodiment, said linker comprises (Gly) $_n$, wherein n is 6, 7, or 8. In one embodiment, said linker comprises (EAAAK) $_n$, wherein n is 1, 2, 3, 4, 5, or 6. In one embodiment, said linker

comprises A(EAAAK)₄ALEA(EAAAK)₄A. In one embodiment, said linker comprises a sequence as shown in any one of SEQ ID NOs: 17-34. In one embodiment, 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, a TGF- β 1 inhibitory peptide or a variant thereof.

[0006] In one embodiment, said TGF β R is transforming growth factor beta receptor II (TGF β RII) or said functional fragment thereof. In one embodiment, said functional fragment of TGF β RII is a TGF β RII extracellular domain (ECD). In one embodiment, said ECD binds TGF- β 1. In one embodiment, said ECD binds TGF- β 3. In one embodiment, said ECD binds TGF- β 1 and TGF- β 3. In one embodiment, said ECD binds TGF- β 1 and TGF- β 3 but not TGF- β 2. In one embodiment, 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. In one embodiment, said TGF- β cytokine trap comprises a sequence as shown in SEQ ID NO: 14, SEQ ID NO: 141, or SEQ ID NO: 142.

[0007] In one embodiment, said TGF- β cytokine trap comprises a sequence as shown in SEQ ID NO: 14. In one embodiment, said antibody (anti-PD1) is immunoglobulin G (IgG) antibody. In one embodiment, said IgG is IgG1, IgG2, IgG3, or IgG4. In one embodiment, said IgG4 comprises a mutation at position 108 of SEQ ID NO 146 or SEQ ID NO: 292. In one embodiment, said mutation is S108P mutation. In one embodiment, said IgG4 is connected to said TGF- β cytokine trap by said linker. In one embodiment, said fragment of said antibody is a Fab, (Fab)₂, (Fab')₂, Fv, (Fv)₂, or scFv.

[0008] In one embodiment, said antibody comprises a variable region of heavy chain (V_H) and a variable region of light chain (V_L) of said antibody. In one embodiment, said linker connects said variable region of heavy chain (V_H) to said TGF- β cytokine trap. In one embodiment, said linker connects said variable region of light chain (V_L) to said TGF- β cytokine trap. In one embodiment, 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. In one embodiment, said second linker comprises a sequence as shown in any one of SEQ ID NOs: 17-34. In one embodiment, 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.

[0009] In one embodiment, 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. In one embodiment, 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. In one embodiment, said variable region of light chain (V_L)

comprises a sequence as shown in any one of SEQ ID NOs: 8-13 and 148. In one embodiment, 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. In one embodiment, 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.

[0010] In one embodiment, said fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 16. In one embodiment, said fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 143. In one embodiment, 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. In one embodiment, 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 a sequence as shown in SEQ ID NO: 13.

[0011] In one embodiment, said fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 145. In one embodiment, said fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 144. In one embodiment, said antibody further comprises fragment crystallizable region (F_C). In one embodiment, said F_C is human F_{C1} , F_{C2} , F_{C3} , F_{C4} , or a fragment thereof. In one embodiment, said F_C further comprises one or more mutations. In one embodiment, said antibody comprises said scFv and said F_C fragment. In one embodiment, said TGF β cytokine trap comprises said anti-TGF β antibody or said antigen binding fragment thereof. In one embodiment, the anti-TGF β antibody comprises V_H encoded by SEQ ID NOs: 166, 168, 169, 171, 173, 175, or 177, and V_L encoded by SEQ ID NOs: 165, 167, 170, 172, 174, 176, or 178.

[0012] In one embodiment, said TGF β cytokine trap comprises said TGF β inhibitory peptides or said variant thereof. In one embodiment, the TGF β inhibitory peptides comprises sequence set forth in any one of SEQ ID NOs. 193-227.

[0013] Provided herein is a polynucleotide encoding the fusion protein disclosed herein.

[0014] Provided herein is an expression vector comprising a polynucleotide encoding the fusion protein disclosed herein, wherein said polynucleotide is operably linked to a promoter. In one embodiment, said promoter is a constitutive promoter, a tissue specific promoter, or an inducible promoter. In one embodiment, said inducible promoter is a small molecule ligand-

inducible two polypeptide ecdysone receptor-based gene switch. In one embodiment, said vector is an adenoviral vector.

[0015] Provided herein is a pharmaceutical composition, comprising: (a) the fusion protein disclosed herein, (b) a polynucleotide encoding the fusion protein disclosed herein, or (c) the expression vector disclosed herein, and (d) a pharmaceutically acceptable excipient.

[0016] Provided herein is a method of treating cancer, comprising: contacting a cell with (a) the fusion protein disclosed herein, (b) a polynucleotide encoding the fusion protein disclosed herein, or (c) the expression vector disclosed herein. In one embodiment, said cell is a cancer cell. In one embodiment, said cell is a mammalian cell.

[0017] Provided herein is 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.

[0018] In one embodiment, said linker comprises (G4S) n , wherein n is 2, 3, 4, 5, or 6. In one embodiment, said linker comprises (Gly) n , wherein n is 6, 7, or 8. In one embodiment, said linker comprises (EAAAK) n , wherein n is 1, 2, 3, 4, 5, or 6. In one embodiment, said linker comprises A(EAAAK) $_4$ LEA(EAAAK) $_4$ A. In one embodiment, said linker comprises a sequence as shown in any one of SEQ ID NOs: 17-34. In one embodiment, said transforming growth factor receptor protein is TGF β RII. In one embodiment, said functional fragment of said TGF β RII is TGF β RII extracellular domain (ECD). In one embodiment, 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. In one embodiment, said TGF- β cytokine trap comprises a sequence as shown in SEQ ID NO: 14, SEQ ID NO: 141, or SEQ ID NO: 142.

[0019] In one embodiment, said antibody is immunoglobulin G (IgG) antibody. In one embodiment, said IgG is IgG1, IgG2, IgG3, or IgG4. In one embodiment, said IgG4 comprises a mutation at position 108 of SEQ ID NO: 146 or SEQ ID NO: 292. In one embodiment, said mutation is S108P mutation. In one embodiment, said fragment of said antibody is a Fab, (Fab) $_2$, (Fab') $_2$, Fv, (Fv) $_2$, or scFv of said antibody. In one embodiment, 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 $_L$). In one

embodiment, said linker connects said variable region of heavy chain (V_H) to said TGF- β cytokine trap. In one embodiment, said linker connects said variable region of light chain (V_L) and to said TGF- β cytokine trap.

[0020] In one embodiment, said variable region of heavy chain (V_H) is connected to said variable region of light chain (V_L) by a second linker. In one embodiment, 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. In one embodiment, 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. In one embodiment, 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

[0021] In one embodiment, said variable region of light chain (V_L) comprises a sequence as shown in any one of SEQ ID NOs: 8-13 and 148. In one embodiment, 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. In one embodiment, 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. In one embodiment, said fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 16.

[0022] In one embodiment, 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. In one embodiment, 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 (V_L) is at least 90% identical to a sequence as shown in SEQ ID NO: 13. In one embodiment, 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. In one embodiment, said fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 145. In one embodiment, said fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 144.

[0023] In one embodiment, said antibody further comprises fragment crystallizable region (F_C). In one embodiment, said F_C is human F_{C1} , F_{C2} , F_{C3} , F_{C4} , or a fragment thereof. In one embodiment, said F_C further comprises one or more mutations. In one embodiment, said antibody comprises said scFv and said F_C fragment. In one embodiment, said cancer is a refractory cancer. In one embodiment, said subject is non-responsive to a treatment with a PD-1 antibody or a CTLA-4 antibody. In one embodiment, the method further comprises

administering one or more additional anti-cancer agent. In one embodiment, said additional anti-cancer agent is a PD-1 inhibitor, PD-L1 inhibitor or a CTLA-4 inhibitor. In one embodiment, said PD-1 inhibitor is an anti-PD-1 antibody or a fragment or a variant thereof.

[0024] In one embodiment, said CTLA-4 inhibitor is an anti-CTLA-4 antibody or a fragment or a variant thereof. In one embodiment, the method further comprises administering one or more cytokines. In one embodiment, said subject is a mammalian subject. In one embodiment, said subject is a human. In one embodiment, 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.

[0025] In one embodiment, the skin cancer is cutaneous squamous-cell carcinoma, melanoma or basal cell cancer. In one embodiment, the lung cancer is non small cell lung cancer (NSLC) or small cell lung cancer (SCLC). In one embodiment, the breast cancer is triple negative breast cancer (TNBC). In one embodiment, the method further comprises administering an effective amount of T cells engineered to express an exogenous receptor. In one embodiment, the exogenous receptor is a chimeric antigen receptor. In one embodiment, the chimeric antigen receptor is an engineered T-cell receptor.

[0026] In one embodiment, 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-a2, 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. In one embodiment, the antigen binding domain comprises a sequence selected from SEQ ID NOs: 37-56. In one embodiment, the antigen binding domain comprises a sequence selected from SEQ ID NOs: 35-36.

[0027] In one embodiment, an effective amount of engineered T-cells is at least 10^2 cells/kg. In one embodiment, an effective amount of engineered T-cells is at least 10^4 cells/kg. In one embodiment, an effective amount of engineered T-cells is at least 10^5 cells/kg. In one embodiment, the engineered T cells further express a cytokine. In one embodiment, the cytokine is a fusion protein comprising IL- 15 and IL- 15R α .

[0028] Provided herein is 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.

[0029] In one embodiment, said fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 294. In one embodiment, said fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 295. In one embodiment, said fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 294. In one embodiment, said fusion protein comprises a sequence as shown in SEQ ID NO: 13 and a sequence as shown in SEQ ID NO: 295.

[0030] Disclosed herein are, *inter alia*, fusion proteins 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 (b) an adenosine deaminase (ADA) protein or a functional fragment thereof; wherein one or more polypeptides of the fusion protein are connected by a linker. In one embodiment, the linker comprises (G4S) $_n$, wherein n is 2, 3, 4, 5, or 6. In some embodiments, the linker comprises (Gly) $_n$, wherein n is 6, 7, or 8. In another embodiment, the linker comprises (EAAAK) $_n$, wherein n is 1, 2, 3, 4, 5, or 6. In certain embodiments, the linker comprises A(EAAAK) $_4$ LEA(EAAAK) $_4$ A. In certain embodiments, the linker comprises a sequence as shown in any one of SEQ ID NOs: 17-34.

[0031] In one embodiment, the adenosine deaminase protein is adenosine deaminase 2 (ADA2) or a mutant thereof or a variant thereof. In another embodiment, the adenosine deaminase (ADA) protein comprises any one of ADA2 mutant 1 (SEQ ID NO: 273), ADA2 mutant 2 (SEQ ID NO: 274), ADA2 mutant 3 (SEQ ID NO 275): , ADA2 mutant 4 (SEQ ID NO: 276), ADA2 mutant 5 (SEQ ID NO: 277), or ADA2 mutant 6 (SEQ ID NO: 278), ADA2 mutant 7 (SEQ ID NO: 279), or wild type ADA2 (SEQ ID NO: 284). In other embodiment, the adenosine deaminase (ADA) protein comprises a sequence at least 80% identical to a sequence as shown in any one of SEQ ID NOs: 284 or 273-279. In certain embodiments, the adenosine deaminase (ADA) protein comprises a sequence as shown in any one of SEQ ID NOs: 284 or 273-279.

[0032] In one embodiment, the antibody is immunoglobulin G (IgG) antibody. In certain embodiments, the IgG is IgG1, IgG2, IgG3, or IgG4. In one embodiment, the IgG4 comprises a mutation at position 108 of SEQ ID NOs: 146 or 292. In some embodiments, the mutation is S108P mutation. In some examples, the fragment of the antibody is a Fab, (Fab)₂, (Fab')₂, F_v, (F_v)₂, or scF_v of the antibody. In one embodiment, the antibody or a fragment of the antibody or a variant of the antibody comprises a variable region of heavy chain (V_H) and a variable region of light chain (V_L). In one embodiment, the linker connects the variable region of heavy chain (V_H) to adenosine deaminase 2 (ADA2) or a mutant thereof or a variant thereof. In another embodiment, the linker connects the variable region of light chain (V_L) to the adenosine deaminase 2 (ADA2) or a mutant thereof or a variant thereof.

[0033] In one example, the variable heavy region (V_H) is connected to the variable light region (V_L) by a second linker. In one embodiment, the 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. In another example, the 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. In a further embodiment, the variable region of heavy chain (V_H) comprises a sequence as shown in any one of SEQ ID NOs: 1-7 and 149-164. In a further embodiment, the variable region of light chain (V_L) comprises a sequence as shown in any one of SEQ ID NOs: 8-13 and 148. In one embodiment, the variable region of heavy chain (V_H) is at least 90% identical to a sequence as shown in SEQ ID NO: 6 (VH6) and the variable region of light chain (V_L) is at least 90% identical to a sequence as shown in SEQ ID NO: 12 (VL5). In an embodiment, the 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.

[0034] In one example, the fusion protein comprises a sequence as shown in SEQ ID NO: 12 (VL5) and a sequence as shown in SEQ ID NO: 280 (VH6 IgG4 (mut)- ADA2 wt). In another example, the fusion protein comprises a sequence as shown in SEQ ID NO: 12 (VL5) and a sequence as shown in SEQ ID NO: 281 (VH6 igG4 (mut) ADA2 mut 7). In one example, the variable region of heavy chain (V_H) is at least 90% identical to a sequence as shown in SEQ ID NO: 7 (VH7) and said variable region of light chain (V_L) is at least 90% identical to a sequence as shown in SEQ ID NO: 13 (VL6). In one example, the variable region of heavy chain (V_H) comprises a sequence as shown in SEQ ID NO: 7 (VH7) and the variable region of light chain (V_L) comprises a sequence as shown in SEQ ID NO: 13 (VL6).

In another example, the fusion protein comprises a sequence as shown in SEQ ID NO: 13 (VL6), and a sequence as shown in SEQ ID NO: 282 (VH7 igG4 (mut) ADA2 wt). In one example, the fusion protein comprises a sequence as shown in SEQ ID NO: 13 (VL6), and a sequence as shown in SEQ ID NO: 283 (VH7 igG4 (mut) ADA2 mut 7).

[0035] Provided herein is a polynucleotide encoding the fusion protein. Further provided herein is an expression vector comprising a polynucleotide encoding the fusion protein of any one of aspects above, wherein the polynucleotide is operably linked to a promoter. In some embodiments, the promoter is a constitutive promoter, a tissue specific promoter, or an inducible promoter. In some embodiments, the inducible promoter is a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch. In some embodiments, the vector is an adenoviral vector.

[0036] Provided herein is a method of treating cancer, comprising: contacting a cell with the fusion protein, a polynucleotide encoding the fusion protein, or the expression vector. In some embodiments, the cell is a cancer cell. In some embodiments, the cell is a mammalian cell.

[0037] Further provided herein are a pharmaceutical compositions comprising the fusion protein; or a polynucleotide encoding the fusion protein; or the expression vector and, a pharmaceutically acceptable excipient.

[0038] Provided herein is a method of treating a subject with a cancer, the method comprising 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 an adenosine deaminase protein or a functional fragment thereof; wherein one or more polypeptides of the fusion protein are connected by a linker. In certain cases, the adenosine deaminase protein is adenosine deaminase 2 (ADA2). In other embodiments, the adenosine deaminase (ADA) protein comprises any one of ADA2 mutant 1 (SEQ ID NO: 273), ADA2 mutant 2 (SEQ ID NO: 274), ADA2 mutant 3 (SEQ ID NO 275): , ADA2 mutant 4 (SEQ ID NO: 276), ADA2 mutant 5 (SEQ ID NO: 277), or ADA2 mutant 6 (SEQ ID NO: 278), ADA2 mutant 7 (SEQ ID NO: 279), or wild type ADA2 (SEQ ID NO: 284).

[0039] In certain cases the linker comprises (G4S)_n, wherein n is 2, 3, 4, 5, or 6. In some embodiments, the linker is (Gly)_n, wherein n is 6, 7, or 8. In some embodiments, the linker comprises (EAAAK)_n, wherein n is 1, 2, 3, 4, 5, or 6. In some embodiments, the linker comprises A(EAAAK)₄ALEA(EAAAK)₄A. In some cases, the linker comprises a sequence as shown in any one of SEQ ID Nos: 17-34.

[0040] Provided herein is a method of treating a subject with a cancer. In certain cases, the 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. In certain cases, the skin cancer is cutaneous squamous-cell carcinoma, melanoma or basal cell cancer. In other cases, the lung cancer is non small cell lung cancer (NSLC) or small cell lung cancer (SCLC). In some cases, the breast cancer is triple negative breast cancer (TNBC).

[0041] In another embodiment, there is a method of treating a subject with a cancer, the method comprising 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 an adenosine deaminase protein or a functional fragment thereof; wherein one or more polypeptides of the fusion protein are connected by a linker. In a further embodiment, the method of treating a subject with cancer further comprises administering an effective amount of T cells engineered to express an exogenous receptor.

[0042] In some cases, the exogenous receptor is a chimeric antigen receptor. In other cases, the chimeric antigen receptor is an engineered T-cell receptor. In one case, 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-a2, 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. In some embodiments, the antigen binding domain comprises sequence selected from SEQ ID NOs: 37-56. In other embodiments, the antigen binding domain comprises sequence selected from SEQ ID NOs: 35-36.

[0043] In one embodiment, an effective amount of engineered T-cells is at least 10^2 cells/kg. In another embodiment, an effective amount of engineered T-cells is at least 10^4 cells/kg. In a further embodiment, the effective amount of engineered T-cells is at least 10^5 cells/kg.

[0044] In a further embodiment, the engineered T cells further express a cytokine. In another embodiment, the cytokine is a fusion protein comprising IL- 15 and IL- 15R α .

[0045] Provided herein is a method of treating cancer in a subject in need thereof comprising 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 an adenosine deaminase protein or a functional fragment thereof; wherein one or more polypeptides of the fusion protein are connected by a linker, and 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.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0046] The features of the present disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:
- [0047] FIG. 1 is a schematic of PD-1/PD-L1 in immunosuppression.
- [0048] FIG. 2 is a schematic of TGF- β in immunosuppression.
- [0049] FIG. 3 shows TGF- β associated gene cluster correlated with metastatic disease and poor prognosis in subset of ovarian cancer patients (enriched in Stage III/IV).
- [0050] FIG. 4A, FIG. 4B, and FIG. 4C show a schematic design of anti-PD1-TGFR2 fusion protein design. In other exemplary embodiments, ADA2 can be fused to anti-PD1.
- [0051] FIG. 5 is a graph showing blockade of PD-1/PD-L1 interaction by anti-PD1(VH6-VL5) IgG1-TGF β R2 and anti-PD1(VH6-VL5) IgG4-TGF β R2.
- [0052] FIG. 6 is a graph showing neutralization of TGF- β 1 isoform signaling by anti-PD1 (VH6-VL5) IgG1-TGF β R2 and anti-PD1 (VH6-VL5) IgG4-TGF β R2.
- [0053] FIG. 7 is a graph showing neutralization of TGF- β 2 isoform by anti-PD1(VH6-VL5) IgG1-TGF β R2 and anti-PD1(VH6-VL5) IgG4-TGF β R2.
- [0054] FIG. 8 is a graph showing neutralization of TGF- β 3 isoform signaling by anti-PD1(VH6-VL5) IgG1-TGF β R2 and anti-PD1(VH6-VL5) IgG4-TGF β R2.
- [0055] FIG. 9A, FIG. 9B, and FIG. 9C are graphs showing enhanced proliferation and IFN- γ production by stimulated PBMCs in the presence of anti-PD1-TGFR2 fusion protein in a dose dependent manner compared to anti-PD1 or control antibodies.
- [0056] FIG. 9D and FIG. 9E are graphs showing PD1 receptor occupancy on CD8⁺ T cells and IFN- γ production respectively when anti-PD1-TGFR2 fusion protein is added to co-culture of PBMC and colorectal cancer (colorectal adenocarcinoma) cell line.

- [0057] FIG. 9F and FIG. 9G are graphs showing PD1 receptor occupancy on CD8⁺ T cells and IFN- γ production respectively when anti-PD1-TGFR2 fusion protein is added to co-culture of PBMC and head and neck cancer (pharyngeal carcinoma) cell line.
- [0058] FIG. 9H and FIG. 9I are graphs depicting TGF- β 1 and TGF- β 2 concentration respectively in supernatant of PBMC and colorectal cancer (colorectal adenocarcinoma) cells co-culture in presence of anti-PD1-TGFR2 fusion protein.
- [0059] FIG. 9J and FIG. 9K are graphs depicting TGF- β 1 and TGF- β 2 concentration respectively in supernatant of PBCM and head and neck cancer (pharyngeal carcinoma) cells co-culture in presence of anti-PD1-TGFR2 fusion protein.
- [0060] FIG. 9L and FIG. 9M are graphs depicting IFN- γ production respectively in 3D spheroid culture of colorectal cancer (colorectal adenocarcinoma) cells or head & neck cancer (pharyngeal carcinoma) cells and PBMC in presence of anti-PD1-TGFR2 fusion protein as compared to anti-PD1 alone.
- [0061] FIGs. 10A-10D show effect of anti-PD1-TGFR2 fusion protein treatment on T cell proliferation and activation in the presence of recombinant TGF- β 1.
- [0062] FIGs. 11A-11F show expression of various cytokines by PBMC in the presence recombinant TGF- β 1 and in the presence of anti-PD1, anti-PD1-TGFR2 fusion protein or control antibodies.
- [0063] FIG. 12A shows the effect of anti-PD1-TGFR2 fusion protein on tumor growth as compared to anti-PD1 alone in a humanized mouse model of colorectal cancer.
- [0064] FIG. 12B shows that treatment with anti-PD1-TGFR2 fusion protein significantly increases CD8⁺ T cell to T_{reg} ratio in tumor in a humanized mouse model of colorectal cancer.
- [0065] FIG. 12C shows the effect of anti-PD1-TGFR2 fusion protein treatment on perforin expression levels as compared to anti-PD1 treatment in a humanized mouse model of colorectal cancer.
- [0066] FIG. 12D and FIG. 12E shows the effect of anti-PD1-TGFR2 fusion protein treatment on TGF- β 1 and TGF- β 2 concentrations as compared to anti-PD1 treatment respectively in a humanized mouse model of colorectal cancer.
- [0067] FIG. 13A shows treatment with anti-PD1-TGFR2 fusion protein significantly improved IFN γ production compared to anti-PD1 treatment in an in vitro model of head and neck cancer. FIGs. 13B-13G show treatment with anti-PD1-TGFR2 fusion protein significantly increases T-cell function as evidenced by expression analysis of various pathway genes.

- [0068] FIG. 14A shows the effect of anti-PD1-TGFR2 fusion protein on tumor growth as compared to anti-PD1 alone in a humanized mouse model of head and neck cancer.
- [0069] FIG. 14B shows survival of tumor bearing mice in humanized mouse model of head and neck cancer when treated with anti-PD1-TGFR2 fusion protein vs. anti-PD1 alone or isotype control.
- [0070] FIG. 14C shows ratio of CD8⁺ T cells to regulatory T cells in tumors of mice treated with anti-PD1-TGFR2 fusion protein in a humanized mouse model of head and neck cancer.
- [0071] FIG. 14D and FIG. 14E shows the effect of anti-PD1-TGFR2 fusion protein on TGF- β 1 and TGF- β 2 concentrations as compared to anti-PD1 alone in a humanized mouse model of head and neck cancer.
- [0072] FIG. 14F shows the effect of anti-PD1-TGFR2 fusion protein on IFN- γ production in a humanized mouse model of head and neck cancer.
- [0073] FIGs. 15A-15B shows the effect of anti- PD1(VH7/VL6)--TGFR2 fusion protein on IFN- γ production and TGF- β 1 concentration in primary colorectal cancer patient samples.
- [0074] FIG. 15C shows the gene expression analysis of primary colorectal cancer patient samples co-cultured with anti- PD1(VH7/VL6)--TGFR2 fusion protein.
- [0075] FIG. 16 shows a graph depicting the results of a cytotoxicity assay of anti- PD1 (VH7/VL6)-TGFR2 fusion protein compared to anti-PD-L1--TGFR2 fusion protein.
- [0076] FIG. 17 is a graph depicting the results of a cytotoxicity assay of anti- PD1(VH7/VL6)-TGFR2 fusion protein in combination with a chimeric receptor antigen (CAR) T cells vs. anti-PD1 in combination with a CAR T cells vs. CAR T cells alone.
- [0077] FIG. 18A and FIG. 18B are graphs depicting the results of a cytotoxicity assay of anti-PD1 (VH6/VL5)-TGFR2 fusion protein in combination with CD33 CAR-T and a cytotoxicity assay of anti-PD1 (VH7/VL6)-TGFR2 fusion protein in combination with CD33 CAR-T respectively.
- [0078] FIG. 19A and FIG. 19B are graphs depicting tumor cell lysis using anti-PD1 (VH6/VL5)-TGFR2 fusion protein and anti-PD1 (VH7/VL6)-TGFR2 fusion protein respectively when co-cultured with NK cells.
- [0079] FIG. 20 is a graph depicting Biacore analysis of simultaneous binding of TGF- β 1 and PD1 by anti-PD1 (VH6/VL5)-TGFR2 fusion proteins using different linkers.
- [0080] FIG. 21 is a graph showing blockade of PD-1/PD-L1 interaction by anti-PD1 IgG4-ADA2.
- [0081] FIG. 22 is a graph showing ADA2 enzymatic activity measured for anti-PD1 hIgG1-ADA2 and anti-PD1 hIgG4-ADA2.

- [0082] FIGs. 23A-23C are graphs showing the effect of various variants of Anti-PD1 and Anti-PD1-ADA2 fusion proteins on PD-L1/PD-1 interaction.
- [0083] FIGs. 24A-24F are graphs showing the enzymatic activity of various variants of Anti-PD1-ADA2 fusion proteins as measured by ADA enzymatic activity.
- [0084] FIG. 25 is a graph showing the enzymatic activity of Anti-PD1-mutADA2 vs. anti-PD1-wtADA2 as measured by ADA enzymatic activity.
- [0085] FIG. 26A-26D are graphs depicting the effect of variants of anti-PD1-wtADA2 on T cell proliferation.
- [0086] FIG. 26E is a graph depicting IFN γ production by anti-PD1-wtADA2 as compared to anti-PD1 or isotype control.
- [0087] FIGs. 27A-27B are graphs depicting the effectiveness of wtADA2 and mutADA2 to reverse adenosine-mediated suppression of T cell proliferation.
- [0088] FIG. 28 is a graph depicting the effect of variants of anti-PD1-ADA2 fusion proteins on the blockade of PD1-PDL1 interaction.
- [0089] FIG. 29 a graph showing the enzymatic activity of Anti-PD1-ADA2-scFv-Fc as measured by ADA enzymatic activity.
- [0090] FIG. 30 is a bar graph showing the enzymatic activity of variants of anti-PD1-ADA2 as measured by ADA enzymatic activity.
- [0091] FIGs. 31A-31B are graphs depicting the effect of anti-PD1-wtADA2 on IFN- γ Production and Proliferation of Tumor-infiltrating Lymphocytes (TILs) in Primary CRC Patient Tumor.
- [0092] FIG. 32 is a graph depicting the effect of anti-PD1 vs. anti-PD1-wtADA2 on tumor volume of a humanized mouse model of lung cancer.
- [0001] FIGs. 33A-33C show a schematic design of anti-PD1- Adenosine deaminase 2 (ADA2) design: anti-PD1-ADA2.

DETAILED DESCRIPTION OF THE DISCLOSURE

- [0093] The following description and examples illustrate embodiments of the present disclosure in detail.
- [0094] It is to be understood that the present disclosure is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are variations and modifications of the present disclosure, which are encompassed within its scope.
- [0095] All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein

have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

[0096] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0097] Although various features of the disclosure can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the present disclosure can be described herein in the context of separate embodiments for clarity, the present disclosure can also be implemented in a single embodiment.

[0098] The following definitions supplement those in the art and are directed to the current application and are not to be imputed to any related or unrelated case, *e.g.*, to any commonly owned patent or application. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present disclosure, the preferred materials and methods are described herein. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

Definitions

[0099] In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

[0100] In this application, the use of “or” means “and/or” unless stated otherwise. The terms “and/or” and “any combination thereof” and their grammatical equivalents as used herein, can be used interchangeably. These terms can convey that any combination is specifically contemplated. Solely for illustrative purposes, the following phrases “A, B, and/or C” or “A, B, C, or any combination thereof” can mean “A individually; B individually; C individually; A and B; B and C; A and C; and A, B, and C.” The term “or” can be used conjunctively or disjunctively, unless the context specifically refers to a disjunctive use.

[0101] Furthermore, use of the term “including” as well as other forms, such as “include,” “includes,” and “included,” is not limiting.

[0102] Reference in the specification to “some embodiments,” “an embodiment,” “one embodiment” or “other embodiments” means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosures.

[0103] As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present disclosure.

[0104] The term “about” in relation to a reference numerical value and its grammatical equivalents as used herein can include the numerical value itself and a range of values plus or minus 10% from that numerical value.

[0105] The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. In another example, the amount “about 10” includes 10 and any amounts from 9 to 11. In yet another example, the term “about” in relation to a reference numerical value can also include a range of values plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% from that value. Alternatively, particularly with respect to biological systems or processes, the term “about” can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term “about” meaning within an acceptable error range for the particular value should be assumed.

[0106] “Polynucleotide” or “oligonucleotide” as used herein refers to a polymeric form of nucleotides or nucleic acids of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double and single stranded DNA, triplex DNA, as well as double and single stranded RNA. It also includes modified, for example, by methylation and/or by capping, and unmodified forms of the polynucleotide. The term is also meant to include molecules that include non-naturally occurring or synthetic nucleotides as well as nucleotide analogs.

[0107] “Transfection,” “transformation,” or “transduction” as used herein refer to the introduction of one or more exogenous polynucleotides into a host cell by using physical or

chemical methods. The polynucleotide sequences and vectors disclosed or contemplated herein can be introduced into a cell by, for example, transfection, transformation, or transduction. Many transfection techniques are known in the art and include, for example, calcium phosphate DNA co-precipitation (see, *e.g.*, Murray E. J. (ed.), *Methods in Molecular Biology*, Vol. 7, *Gene Transfer and Expression Protocols*, Humana Press (1991)); DEAE-dextran; electroporation; cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, *Nature*, 346: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash et al., *Mol. Cell Biol.*, 7: 2031-2034 (1987)). Phage or viral vectors can be introduced into host cells, after growth of infectious particles in suitable packaging cells, many of which are commercially available.

[0108] “Polypeptide”, “peptide”, and their grammatical equivalents as used herein refer to a polymer of amino acid residues. The polypeptide can optionally include glycosylation or other modifications typical for a given protein in a given cellular environment. Polypeptides and proteins disclosed herein (including functional portions and functional variants thereof) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α -amino n-decanoic acid, homoserine, S-acetylaminoethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β -phenylserine β -hydroxyphenylalanine, phenylglycine, α -naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α -aminocyclopentane carboxylic acid, α -aminocyclohexane carboxylic acid, α -aminocycloheptane carboxylic acid, α -(2-amino-2-norbornane)-carboxylic acid, α,γ -diaminobutyric acid, α,β -diaminopropionic acid, homophenylalanine, and α -tert-butylglycine. The present disclosure further contemplates that expression of polypeptides or proteins described herein in an engineered cell can be associated with post-translational modifications of one or more amino acids of the polypeptide or protein. Non-limiting examples of post-translational modifications include phosphorylation, acylation including acetylation and formylation, glycosylation (including N-linked and O-linked), amidation, hydroxylation, alkylation including methylation and ethylation, ubiquitylation, addition of pyrrolidone carboxylic acid, formation of disulfide bridges, sulfation, myristoylation, palmitoylation, isoprenylation, farnesylation, geranylation, glypiation, lipoylation and iodination.

[0109] The terms “identical” and its grammatical equivalents as used herein or “sequence identity” in the context of two nucleic acid sequences or amino acid sequences of polypeptides refer to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window. A “comparison window”, as used herein, refers to a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence can be compared to a reference sequence of the same number of contiguous positions after the two sequences are aligned optimally. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman, *Adv. Appl. Math.*, 2:482 (1981); by the alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.*, 48:443 (1970); by the search for similarity method of Pearson and Lipman, *Proc. Nat. Acad. Sci U.S.A.*, 85:2444 (1988); by computerized implementations of these algorithms (including, but not limited to CLUSTAL in the PC/Gene program by Intelligentics, Mountain View Calif., GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis., U.S.A.); the CLUSTAL program is well described by Higgins and Sharp, *Gene*, 73:237-244 (1988) and Higgins and Sharp, *CABIOS*, 5:151-153 (1989); Corpet et al., *Nucleic Acids Res.*, 16:10881-10890 (1988); Huang et al., *Computer Applications in the Biosciences*, 8:155-165 (1992); and Pearson et al., *Methods in Molecular Biology*, 24:307-331 (1994). Alignment is also often performed by inspection and manual alignment. In one class of embodiments, the polypeptides herein are at least 80%, 85%, 90%, 98% 99% or 100% identical to a reference polypeptide, or a fragment thereof, *e.g.*, as measured by BLASTP (or CLUSTAL, or any other available alignment software) using default parameters. Similarly, nucleic acids can also be described with reference to a starting nucleic acid, *e.g.*, they can be 50%, 60%, 70%, 75%, 80%, 85%, 90%, 98%, 99% or 100% identical to a reference nucleic acid or a fragment thereof, *e.g.*, as measured by BLASTN (or CLUSTAL, or any other available alignment software) using default parameters. When one molecule is said to have certain percentage of sequence identity with a larger molecule, it means that when the two molecules are optimally aligned, the percentage of residues in the smaller molecule finds a match residue in the larger molecule in accordance with the order by which the two molecules are optimally aligned.

[0110] The term “substantially identical” and its grammatical equivalents as applied to nucleic acid or amino acid sequences mean that a nucleic acid or amino acid sequence comprises a

sequence that has at least 90% sequence identity or more, at least 95%, at least 98% and at least 99%, compared to a reference sequence using the programs described above, *e.g.*, BLAST, using standard parameters. For example, the BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1992)). Percentage of sequence identity is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window can comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. In some embodiments, the substantial identity exists over a region of the sequences that is at least about 50 residues in length, over a region of at least about 100 residues, and in some embodiments, the sequences are substantially identical over at least about 150 residues. In some embodiments, the sequences are substantially identical over the entire length of the coding regions.

[0111] “Homology” is generally inferred from sequence identity between two or more nucleic acids or proteins (or sequences thereof). The precise percentage of identity between sequences that is useful in establishing homology varies with the nucleic acid and protein at issue, but as little as 25% sequence identity is routinely used to establish homology. Higher levels of sequence identity, *e.g.*, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% or more can also be used to establish homology. Methods for determining sequence identity percentages (*e.g.*, BLASTP and BLASTN using default parameters) are described herein and are generally available. Nucleic acids and/or nucleic acid sequences are “homologous” when they are derived, naturally or artificially, from a common ancestral nucleic acid or nucleic acid sequence. Proteins and/or protein sequences are “homologous” when their encoding DNAs are derived, naturally or artificially, from a common ancestral nucleic acid or nucleic acid sequence. The homologous molecules can be termed “homologs.” For example, any naturally occurring proteins can be modified by any available mutagenesis

method. When expressed, this mutagenized nucleic acid encodes a polypeptide that is homologous to the protein encoded by the original nucleic acid.

[0112] The term “isolated” and its grammatical equivalents as used herein refer to the removal of a nucleic acid from its natural environment. The term “purified” and its grammatical equivalents as used herein refer to a molecule or composition, whether removed from nature (including genomic DNA and mRNA) or synthesized (including cDNA) and/or amplified under laboratory conditions, that has been increased in purity, wherein “purity” is a relative term, not “absolute purity.” It is to be understood, however, that nucleic acids and proteins can be formulated with diluents or adjuvants and still for practical purposes be isolated. For example, nucleic acids typically are mixed with an acceptable carrier or diluent when used for introduction into cells. The term “substantially purified” and its grammatical equivalents as used herein refer to a nucleic acid sequence, polypeptide, protein or other compound which is essentially free, *i.e.*, is more than about 50% free of, more than about 70% free of, more than about 90% free of, the polynucleotides, proteins, polypeptides and other molecules that the nucleic acid, polypeptide, protein or other compound is naturally associated with.

[0113] An “expression vector” or “vector” is any genetic element, *e.g.*, a plasmid, chromosome, virus, transposon, behaving either as an autonomous unit of polynucleotide replication within a cell. (*i.e.* capable of replication under its own control) or being rendered capable of replication by insertion into a host cell chromosome, having attached to it another polynucleotide segment, so as to bring about the replication and/or expression of the attached segment. Suitable vectors include, but are not limited to, plasmids, transposons, bacteriophages and cosmids. Vectors can contain polynucleotide sequences which are necessary to effect ligation or insertion of the vector into a desired host cell and to effect the expression of the attached segment. Such sequences differ depending on the host organism; they include promoter sequences to effect transcription, enhancer sequences to increase transcription, ribosomal binding site sequences and transcription and translation termination sequences. Alternatively, expression vectors can be capable of directly expressing nucleic acid sequence products encoded therein without ligation or integration of the vector into host cell DNA sequences. In some embodiments, the vector is an “episomal expression vector” or “episome,” which is able to replicate in a host cell, and persists as an extrachromosomal segment of DNA within the host cell in the presence of appropriate selective pressure (see, *e.g.*, Conese et al., *Gene Therapy*, 11:1735-1742 (2004)). Representative commercially available episomal expression vectors include, but are not limited to, episomal plasmids that

utilize Epstein Barr Nuclear Antigen 1 (EBNA1) and the Epstein Barr Virus (EBV) origin of replication (oriP). The vectors pREP4, pCEP4, pREP7, and pcDNA3.1 from Invitrogen (Carlsbad, Calif.) and pBK-CMV from Stratagene (La Jolla, Calif.) represent non-limiting examples of an episomal vector that uses T-antigen and the SV40 origin of replication in lieu of EBNA1 and oriP. Vector also can comprise a selectable marker gene.

[0114] The term “selectable marker gene” as used herein refers to a nucleic acid sequence that allows cells expressing the nucleic acid sequence to be specifically selected for or against, in the presence of a corresponding selective agent. Suitable selectable marker genes are known in the art and described in, *e.g.*, International Patent Application Publications WO 1992/08796 and WO 1994/28143; Wigler et al., Proc. Natl. Acad. Sci. USA, 77: 3567 (1980); O’Hare et al., Proc. Natl. Acad. Sci. USA, 78: 1527 (1981); Mulligan & Berg, Proc. Natl. Acad. Sci. USA, 78: 2072 (1981); Colberre-Garapin et al., J. Mol. Biol., 150:1 (1981); Santerre et al., Gene, 30: 147 (1984); Kent et al., Science, 237: 901-903 (1987); Wigler et al., Cell, 11: 223 (1977); Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA, 48: 2026 (1962); Lowy et al., Cell, 22: 817 (1980); and U.S. Pat. Nos. 5,122,464 and 5,770,359.

[0115] The term “coding sequence” as used herein refers to a segment of a polynucleotide that encodes for protein or polypeptide. The region or sequence is bounded nearer the 5’ end by a start codon and nearer the 3’ end with a stop codon. Coding sequences can also be referred to as open reading frames.

[0116] The term “operably linked” as used herein refers to refers to the physical and/or functional linkage of a DNA segment to another DNA segment in such a way as to allow the segments to function in their intended manners. A DNA sequence encoding a gene product is operably linked to a regulatory sequence when it is linked to the regulatory sequence, such as, for example, promoters, enhancers and/or silencers, in a manner which allows modulation of transcription of the DNA sequence, directly or indirectly. For example, a DNA sequence is operably linked to a promoter when it is ligated to the promoter downstream with respect to the transcription initiation site of the promoter, in the correct reading frame with respect to the transcription initiation site and allows transcription elongation to proceed through the DNA sequence. An enhancer or silencer is operably linked to a DNA sequence coding for a gene product when it is ligated to the DNA sequence in such a manner as to increase or decrease, respectively, the transcription of the DNA sequence. Enhancers and silencers can be located upstream, downstream or embedded within the coding regions of the DNA sequence. A DNA for a signal sequence is operably linked to DNA coding for a polypeptide if the signal sequence is expressed as a pre-protein

that participates in the secretion of the polypeptide. Linkage of DNA sequences to regulatory sequences is typically accomplished by ligation at suitable restriction sites or via adapters or linkers inserted in the sequence using restriction endonucleases known to one of skill in the art.

[0117] The term “induce”, “induction” and its grammatical equivalents as used herein refer to an increase in nucleic acid sequence transcription, promoter activity and/or expression brought about by a transcriptional regulator, relative to some basal level of transcription.

[0118] The term “transcriptional regulator” refers to a biochemical element that acts to prevent or inhibit the transcription of a promoter-driven DNA sequence under certain environmental conditions (*e.g.*, a repressor or nuclear inhibitory protein), or to permit or stimulate the transcription of the promoter-driven DNA sequence under certain environmental conditions (*e.g.*, an inducer or an enhancer).

[0119] The term “enhancer” as used herein, refers to a DNA sequence that increases transcription of, for example, a nucleic acid sequence to which it is operably linked. Enhancers can be located many kilobases away from the coding region of the nucleic acid sequence and can mediate the binding of regulatory factors, patterns of DNA methylation, or changes in DNA structure. A large number of enhancers from a variety of different sources are well known in the art and are available as or within cloned polynucleotides (from, *e.g.*, depositories such as the ATCC as well as other commercial or individual sources). A number of polynucleotides comprising promoters (such as the commonly-used CMV promoter) also comprise enhancer sequences. Enhancers can be located upstream, within, or downstream of coding sequences. The term “Ig enhancers” refers to enhancer elements derived from enhancer regions mapped within the immunoglobulin (Ig) locus (such enhancers include for example, the heavy chain (μ) 5' enhancers, light chain (κ) 5' enhancers, κ and μ intronic enhancers, and 3' enhancers (see generally Paul W. E. (ed), *Fundamental Immunology*, 3rd Edition, Raven Press, New York (1993), pages 353-363; and U.S. Pat. No. 5,885,827).

[0120] The term “promoter” refers to a region of a polynucleotide that initiates transcription of a coding sequence. Promoters are located near the transcription start sites of genes, on the same strand and upstream on the DNA (towards the 5' region of the sense strand). Some promoters are constitutive as they are active in all circumstances in the cell, while others are regulated becoming active in response to specific stimuli, *e.g.*, an inducible promoter. The term “promoter activity” and its grammatical equivalents as used herein refer to the extent of expression of nucleotide sequence that is operably linked to the promoter whose activity is

being measured. Promoter activity can be measured directly by determining the amount of RNA transcript produced, for example by Northern blot analysis or indirectly by determining the amount of product coded for by the linked nucleic acid sequence, such as a reporter nucleic acid sequence linked to the promoter.

[0121] “Inducible promoter” as used herein refers to a promoter which is induced into activity by the presence or absence of transcriptional regulators, *e.g.*, biotic or abiotic factors.

Inducible promoters are useful because the expression of genes operably linked to them can be turned on or off at certain stages of development of an organism or in a particular tissue.

Non-limiting examples of inducible promoters include alcohol-regulated promoters, tetracycline-regulated promoters, steroid-regulated promoters, metal-regulated promoters, pathogenesis-regulated promoters, temperature-regulated promoters and light-regulated promoters. The inducible promoter can be part of a gene switch or genetic switch.

[0122] “T cell” or “T lymphocyte” as used herein is a type of lymphocyte that plays a central role in cell-mediated immunity. They can be distinguished from other lymphocytes, such as B cells and natural killer cells (NK cells), by the presence of a T-cell receptor (TCR) on the cell surface.

[0123] The term “antibody”, also known as immunoglobulin (Ig), as used herein can be monoclonal or polyclonal antibodies. The term “monoclonal antibodies,” as used herein, refers to antibodies that are produced by a single clone of B-cells and bind to the same epitope. In contrast, “polyclonal antibodies” refer to a population of antibodies that are produced by different B-cells and bind to different epitopes of the same antigen. The antibodies can be from any animal origin. An antibody can be IgG (including IgG1, IgG2, IgG3, and IgG4), IgA (including IgA1 and IgA2), IgD, IgE, or IgM, and IgY. In some embodiments, the antibody can be whole antibodies, including single-chain whole antibodies. In some embodiments, the antibody can be a fragment of an antibody, which can include, but are not limited to, a Fab, a Fab’, a F(ab’)₂, a Fd (consisting of V_H and CH1), a Fv fragment (consisting of V_H and V_L), a single-chain variable fragment (scFv), a single-chain antibody, a disulfide-linked variable fragment (dsFv), and fragments comprising either a V_L or V_H domain. A whole antibody typically consists of four polypeptides: two identical copies of a heavy (H) chain polypeptide and two identical copies of a light (L) chain polypeptide. Each of the heavy chains contains one N-terminal variable (V_H) region and three C-terminal constant (CH1, CH2 and CH3) regions, and each light chain contains one N-terminal variable (V_L) region and one C-terminal constant (C_L) region. The variable regions of each pair of light and heavy chains form the antigen binding site of an antibody.

The V_H and V_L regions have a similar general structure, with each region comprising four framework regions, whose sequences are relatively conserved. The framework regions are connected by three complementarity determining regions (CDRs). The three CDRs, known as CDR1, CDR2, and CDR3, form the “hypervariable region” of an antibody, which is responsible for antigen binding. These particular regions have been described by Kabat et al., J. Biol. Chem. 252, 6609-6616 (1977) and Kabat et al., Sequences of protein of immunological interest. (1991), by Chothia et al., J. Mol. Biol. 196:901-917 (1987), and by MacCallum et al., J. Mol. Biol. 262:732-745 (1996), all of which are herein incorporated by reference in their entireties, where the definitions include overlapping or subsets of amino acid residues when compared against each other. Preferably, the term “CDR” is a CDR as defined by Kabat, based on sequence comparisons. CDRH1, CDRH2 and CDRH3 denote the heavy chain CDRs, and CDRL1, CDRL2 and CDRL3 denote the light chain CDRs.

[0124] The terms “fragment of an antibody,” “antibody fragment,” “fragment of an antibody,” “antigen-binding portion” or its grammatical equivalents are used interchangeably herein to mean one or more fragments or portions of an antibody that retain the ability to specifically bind to an antigen (see, generally, Holliger et al., Nat. Biotech., 23(9):1126-1129 (2005)). The antibody fragment desirably comprises, for example, one or more CDRs, the variable region (or portions thereof), the constant region (or portions thereof), or combinations thereof. Non-limiting examples of antibody fragments include (1) a Fab fragment, which is a monovalent fragment consisting of the V_L , V_H , C_L , and $CH1$ domains; (2) a $F(ab')_2$ fragment, which is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the stalk region; (3) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; (4) a single chain Fv (scFv), which is a monovalent molecule consisting of the two domains of the Fv fragment (*i.e.*, V_L and V_H) joined by a linker which enables the two domains to be synthesized as a single polypeptide chain (see, *e.g.*, Bird et al., Science, 242: 423-426 (1988); Huston et al., Proc. Natl. Acad. Sci. USA, 85: 5879-5883 (1988); and Osbourn et al., Nat. Biotechnol., 16: 778 (1998)) and (5) a diabody, which is a dimer of polypeptide chains, wherein each polypeptide chain comprises a V_H connected to a V_L by a peptide linker that is too short to allow pairing between the V_H and V_L on the same polypeptide chain, thereby driving the pairing between the complementary domains on different V_H - V_L polypeptide chains to generate a dimeric molecule having two functional antigen binding sites. Antibody fragments are known in the art and are described in more detail in, *e.g.*, U.S. Patent 8,603,950.

- [0125] “Antigen recognition moiety,” “antigen recognition domain,” “antigen binding domain,” or “antigen binding region” refers to a molecule or portion of a molecule that specifically binds to an antigen. In one embodiment, the antigen recognition moiety is an antibody, antibody like molecule or fragment thereof.
- [0126] The term “conservative amino acid substitution” or “conservative mutation” refers to the replacement of one amino acid by another amino acid with a common property. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz, G. E. and Schirmer, R. H., Principles of Protein Structure, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids can be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz, G. E. and Schirmer, R. H., *supra*). Examples of conservative mutations include amino acid substitutions of amino acids within the sub-groups above, for example, lysine for arginine and vice versa such that a positive charge can be maintained; glutamic acid for aspartic acid and vice versa such that a negative charge can be maintained; serine for threonine such that a free –OH can be maintained; and glutamine for asparagine such that a free –NH₂ can be maintained. Alternatively or additionally, the functional variants can comprise the amino acid sequence of the reference protein with at least one non-conservative amino acid substitution.
- [0127] The term “non-conservative mutations” involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with, or inhibit the biological activity of, the functional variant. The non-conservative amino acid substitution can enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the homologous parent protein.
- [0128] The term “proliferative disease” as referred to herein refers to a unifying concept in which excessive proliferation of cells and/or turnover of cellular matrix contributes significantly to the pathogenesis of the disease, including cancer. In some embodiments, the proliferative disease is cancer.
- [0129] “Patient” or “subject” as used herein refers to a mammalian subject diagnosed with or suspected of having or developing a proliferative disorder such as cancer. In some embodiments, the term “patient” refers to a mammalian subject with a higher than average

likelihood of developing a proliferative disorder such as cancer. Exemplary patients can be humans, apes, dogs, pigs, cattle, cats, horses, goats, sheep, rodents and other mammals that can benefit from the therapies disclosed herein. Exemplary human patients can be male and/or female. "Patient in need thereof" or "subject in need thereof" is referred to herein as a patient diagnosed with or suspected of having a disease or disorder, for instance, but not restricted to cancer.

[0130] "Administering" is referred to herein as providing one or more compositions described herein to a patient or a subject. By way of example and not limitation, composition administration, *e.g.*, injection, can be performed by intravenous (i.v.) injection, subcutaneous (s.c.) injection, intradermal (i.d.) injection, intraperitoneal (i.p.) injection, or intramuscular (i.m.) injection. One or more such routes can be employed. Parenteral administration can be, for example, by bolus injection or by gradual perfusion over time. Alternatively, or concurrently, administration can be by the oral route. Additionally, administration can also be by surgical deposition of a bolus or pellet of cells, or positioning of a medical device. In an embodiment, a composition of the present disclosure can comprise engineered cells or host cells expressing nucleic acid sequences described herein, or a vector comprising at least one nucleic acid sequence described herein, in an amount that is effective to treat or prevent proliferative disorders. A pharmaceutical composition can comprise a target cell population as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions can comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives.

[0131] As used herein, the term "treatment", "treating", or its grammatical equivalents refers to obtaining a desired pharmacologic and/or physiologic effect. In embodiments, the effect is therapeutic, *i.e.*, the effect partially or completely cures a disease and/or adverse symptom attributable to the disease. In some embodiments, the term "treating" can include "preventing" a disease or a condition.

[0132] As used herein, a "treatment interval" refers to a treatment cycle, for example, a course of administration of a therapeutic agent that can be repeated, *e.g.*, on a regular schedule. In embodiments, a dosage regimen can have one or more periods of no administration of the therapeutic agent in between treatment intervals. For example, a treatment interval can

include one dose of a fusion protein administered in combination with (prior, concurrently or after) administration of a second therapeutic agent, e.g. CAR-T cells.

[0133] The terms “administered in combination” or “co-administration” or “co-administering” or “co-providing” as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, e.g., the two or more treatments are delivered after the subject has been diagnosed with the disorder and before the disorder has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as “simultaneous” or “concurrent delivery”. In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

[0134] In some embodiments, the first treatment and second treatment can be administered simultaneously (e.g., at the same time), in the same or in separate compositions, or sequentially. Sequential administration refers to administration of one treatment before (e.g., immediately before, less than 5, 10, 15, 30, 45, 60 minutes; 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 48, 72, 96 or more hours; 4, 5, 6, 7, 8, 9 or more days; 1, 2, 3, 4, 5, 6, 7, 8 or more weeks before) administration of an additional, e.g., secondary, treatment. The order of administration of the first and secondary treatment can also be reversed.

[0135] The term “therapeutically effective amount”, “therapeutic amount”, “immunologically effective amount”, “anti-tumor effective amount”, “tumor-inhibiting effective amount” or its grammatical equivalents refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. The therapeutically effective amount can vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of a composition described herein to elicit a desired response in one or more

subjects. The precise amount of the compositions of the present disclosure to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject).

[0136] Alternatively, the pharmacologic and/or physiologic effect of administration of one or more compositions described herein to a patient or a subject of can be “prophylactic,” *i.e.*, the effect completely or partially prevents a disease or symptom thereof. A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired prophylactic result (*e.g.*, prevention of disease onset).

Programmed Cell Death Protein and other check point inhibitors

[0137] Programmed cell death protein 1, also known as PD-1 or CD279 (cluster of differentiation 279), is an immune checkpoint protein. The PD-1/PD-L1 signaling axis can promote tumor mediated immune evasion. In some cases, PDL-1 can be over-expressed by tumor cells, accessory cells, such as myeloid-derived suppressor cell (MDSCs), tumor associated macrophages (TAMs), antigen presenting cells (APCs), in the tumor microenvironment. In some cases, PD-1 can be upregulated by “exhausted” T cells and can signal to suppress effector T cells function upon binding to its ligand (PDL-1, PDL2, and CD80). Blockade of the PD-1/PD-L1 pathway by anti-PD-1 or anti-PD-L1 can restore the function of exhausted T cells and promote tumor cell killing (FIG. 1).

[0138] In some embodiments, the fusion protein comprising a PD-1 inhibitor and TGF- β trap can comprise, but not limited to, a full length nivolumab (anti-PD-1), MK-3945 (anti-PD-1), pembrolizumab (anti-PD-1), pidilizumab (anti-PD-1), REGN2810 (anti-PD-1), AMP-224 (anti-PD-1), MEDI0680 (anti-PD-1), PDR001 (anti-PD-1), CT-001 (anti-PD-1), or a functional fragment or a variant thereof. In some embodiments, the PD-1 inhibitor is nivolumab. In some embodiments, the PD-1 inhibitor is pembrolizumab.

Fusion proteins

[0139] In some embodiments, the fusion protein or a fragment thereof or a variant thereof provided herein comprises the PD-1 inhibitor or antibody fused to a cytokine trap via a linker.

In some embodiments, the PD-1 inhibitor can be an antibody or a fragment of the antibody or a variant of the antibody that targets PD-1. In some embodiments, the fusion protein comprising

prembrolizumab can be fused to a cytokine trap (*e.g.*, TGF- β trap). In some embodiments, the fusion protein comprising nivolumab can be fused to a cytokine trap (*e.g.*, TGF- β trap).

[0140] In some embodiments, the fusion protein or a fragment thereof or a variant thereof described herein comprises a cytokine trap as described above and an antibody, a fragment or a variant of the antibody that targets immune checkpoint genes. In some embodiments, an antibody or a fragment of the antibody or a variant of the antibody that target immune checkpoints, such as cytotoxic T lymphocyte associated protein-4 (CTLA-4) and programmed cell death- ligand-1 (PDL1), can be fused to a TGF- β trap molecule via a linker. In some embodiments, the PD-L1 inhibitor is atezolizumab.

[0141] In some embodiments, the fusion protein or a fragment thereof or a variant thereof provided herein comprises the PD1 inhibitor or antibody fused to an adenosine deaminase (*e.g.*, ADA2) described herein or a functional variant or derivative thereof.

Cytokine Trap

[0142] Cytokines have an impact on many biological processes. Inhibiting cytokines can have clinical benefits for example, in cancer. Several cytokines have been shown to be causative agents in a variety of diseases. Such cytokines, include but are not limited to IL-1, IL-4, IL-6, TNF- α , TGF-beta and its various isoforms. The term “cytokine trap” as used herein refers to blockers or neutralizers of cytokine action. Examples of such cytokine traps can include but are not limited to extracellular domains of the cytokine receptors, antibodies that bind to cytokines, and peptides that bind to the cytokines (*e.g.* inhibitory peptides). In one embodiment, the cytokine is TGF- β . In one embodiment, the cytokine is TGF- β 1. In one embodiment, the cytokine is TGF- β 3. In one embodiment, the cytokine is TGF- β 1 and TGF- β 3. In further embodiments, cytokine traps that target TGF- β (*e.g.* TGF- β trap) can include the extracellular domain of TGF- β RII or its variants thereof (for example, SEQ ID NO 141 and 142), anti- TGF- β antibodies and inhibitory peptides of TGF- β 1, TGF- β 2 and/or TGF- β 3.

Transforming Growth Factor

[0143] Transforming growth factor- β (TGF- β) is a multifunctional set of peptides that can control proliferation, differentiation, and other functions in many cell types. TGF- β can act synergistically with TGF- α in inducing transformation. It also can act as a negative autocrine growth factor. Dysregulation of TGF- β activation and signaling can result in apoptosis. Many cells can synthesize TGF- β and almost all of them have specific receptors

for this peptide. TGF- β 1, TGF- β 2, and TGF- β 3 all can function through the same receptor signaling systems. TGF- β 1 can play an important role in controlling the immune system, and can show different activities on different types of cell, or cells at different developmental stages. Most immune cells (or leukocytes) can secrete TGF- β 1. TGF- β 1 is a peptide of 112 amino acid residues derived by proteolytic cleavage from the C-terminal of a precursor protein. TGF- β , a small secreted polypeptide, can signal through the type II serine/threonine kinase dimeric receptor (TGF β RII) that can recruit and phosphorylate the type I dimeric receptor (TGF β RI). TGF β RI can phosphorylate and activate SMADs which can be transcription factors regulating genes involved in cell proliferation, differentiation, apoptosis and growth. Many advanced stage cancers are known to over-express both the TGF- β and TGF β R promoting aggressive tumor formation. Inhibiting the TGF β signaling pathway can be a key therapeutic strategy in treating cancer.

[0144] Some T cells (*e.g.*, regulatory T cells) can release TGF- β 1 to inhibit the actions of other T cells. IL-1 and IL-2 dependent proliferation of activated T cells, and the activation of quiescent helper T cells and cytotoxic T cells can be prevented by the activity of TGF- β 1. Similarly, TGF- β 1 can inhibit the secretion and activity of many other cytokines including, but not limited to, interferon- γ , tumor necrosis factor alpha (TNF- α) and various interleukins. It can also decrease the expression levels of cytokine receptors, such as the IL-2 receptor, to down-regulate the activity of immune cells. However, TGF- β 1 can also increase the expression of certain cytokines in T cells and can promote their proliferation, particularly if the cells are immature (FIG. 2).

[0145] TGF- β 1 can have similar effects on B cells that vary according to the differentiation state of the cell. It can inhibit proliferation and can stimulate apoptosis of B cells, and can play a role in controlling the expression of antibody, transferrin, and MHC class II proteins on immature and mature B cells.

[0146] The effects of TGF- β 1 on macrophages and monocytes can be predominantly suppressive; this cytokine can inhibit the proliferation of these cells and can prevent their production of reactive oxygen (*e.g.*, superoxide (O_2^-)) and nitrogen (*e.g.*, nitric oxide (NO)) intermediates. However, as with other cell types, TGF- β 1 can also have the opposite effect on cells of myeloid origin. For example, TGF- β 1 can act as a chemoattractant, directing an immune response to some pathogens; macrophages and monocytes can respond to low levels of TGF- β 1 in a chemotactic manner. Furthermore, the expression of monocytic cytokines (including IL-1 α , IL-1 β , and TNF- α), and phagocytic killing by macrophages can be increased by the action of TGF- β 1 (FIG. 2).

[0147] Transforming Growth Factor- Beta III (TGF- β 3), a subset of a cytokine family, is responsible for a plethora of functions including cellular proliferation, embryogenesis, immune system regulation, and differentiation.

Transforming Growth Factor- β Receptor II (TGF β RII)

[0148] TGF- β receptors (TGF β R) are single pass serine/threonine kinase receptors. They can exist in several different isoforms that can be homodimeric or heterodimeric. The number of characterized ligands in the TGF- β superfamily can far exceed the number of known receptors, suggesting the promiscuity between the ligand and receptor interactions. Three TGF- β superfamily receptors (TGF β R) specific for TGF- β can be distinguished by their structural and functional properties. TGF β RI (ALK5) and TGF β RII can have similar ligand-binding affinities and can be distinguished from each other only by peptide mapping. Both TGF β RI and TGF β RII can have a high affinity for TGF- β 1 and low affinity for TGF- β 2. TGF β RIII (β -glycan) can have a high affinity for both homodimeric TGF- β 1 and TGF- β 2 and in addition the heterodimer TGF- β 1,2. The TGF β receptors can also bind to TGF- β 3. By “TGF β RII” or “TGF β Receptor II” is meant a polypeptide having the wild-type human TGF β Receptor Type 2 Isoform A sequence (e.g., the amino acid sequence of NCBI Reference Sequence (RefSeq) Accession No. NP_001020018 (SEQ ID NO: 289), or a polypeptide having the wild-type human TGF β Receptor Type 2 Isoform B sequence (e.g., the amino acid sequence of NCBI RefSeq Accession No. NP_003233 (SEQ ID NO: 290) or having a sequence substantially identical the amino acid sequence of SEQ ID NO: 289 or of SEQ ID NO: 290. The TGF β RII may retain at least 0.1%, 0.5%, 1%, 5%, 10%, 25%, 35%, 50%, 75%, 90%, 95%, or 99% of the TGF β -binding activity of the wild-type sequence. The polypeptide of expressed TGF β RII lacks the signal sequence.

[0149] TGF- β 1 can reduce the efficacy of the MHC II in astrocytes and dendritic cells, which in turn can decrease the activation of appropriate helper T cell populations. TGF- β 1 can promote tumor growth as cancer progresses and in some embodiments, does not suppress inflammatory cells responses but can promote regulatory T cell function. TGF- β 1 can be produced by tumor cells, tumor-associated fibroblast cells, regulatory T cells and immature myeloid cells. TGF- β 1 can inhibit T cell priming and promote an exhausted phenotype. TGF- β 1 can suppress the anti-tumor activity of innate immune cell populations including natural killer cells, macrophages and dendritic cells. TGF- β receptor II can be upregulated by tumor-associated myeloid cells and can promote metastasis.

TGF- β trap Fusion Proteins or Fragments or Variant Thereof

[0150] Provided herein is a fusion protein or a fragment or a variant thereof comprising an immune checkpoint inhibitor, such as a PD-1 inhibitor or antibody, and a cytokine trap that can neutralize the cytokine (for instance, TGF- β). In certain cases, a cytokine trap can be a TGF- β trap (also referred to as TGF- β RII or fragment or variant thereof) that comprises SEQ ID NO. 142. Examples of TGF- β trap can include, but are not limited to, extracellular domain (ECD) of the receptor (*e.g.*, TGF β RII) or a functional variant or derivative thereof, TGF- β inhibitory peptides (for instance, SEQ ID NO. 193-227), or an anti-TGF- β antibodies. In some embodiments, the anti-TGF- β antibody comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence selected from any one of SEQ ID NOs: 166, 168, 169, 171, 173, 175, or 177. In some embodiments, the anti-TGF- β antibody comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence selected from any one of SEQ ID NOs: 165, 167, 170, 172, 174, 176, or 178. In certain embodiments, a TGF- β trap can specifically bind or have high affinity for TGF- β 1 or TGF- β 2 or TGF- β 3. In other embodiments, a TGF- β trap can specifically bind or have high affinity for TGF- β 1, TGF- β 2 and TGF- β 3. In other embodiments, a TGF- β trap can specifically bind or have high affinity for TGF- β 1 and TGF- β 3. In further embodiments, a TGF- β trap can have low affinity for or not bind TGF- β 2.

[0151] The fusion protein or a fragment or a variant thereof provided herein (*e.g.*, PD-1 inhibitor or antibody fused to a cytokine trap such as a TGF- β trap) can elicit a synergistic anti-tumor effect due to the simultaneous blockade of the interaction between *e.g.*, PD-L1 on tumor cells and PD-1 on immune cells, and the neutralization of *e.g.*, TGF- β in the tumor microenvironment. Without being bound by theory, this effect is obtained from simultaneous blocking the two major immune escape mechanisms and the targeted depletion of the TGF- β in the tumor microenvironment by a single molecular entity. This depletion can be achieved by one or more of the following: (1) anti-PD-1 targeting of tumor cells; (2) binding of the TGF- β in the tumor microenvironment by the TGF- β trap (*e.g.*, TGF β RII); and/or (3) destruction of the bound TGF- β through the PD-L1 receptor mediated endocytosis. The fusion protein or a fragment or a variant thereof provided herein (*e.g.*, PD-1 inhibitor or antibody fused to a cytokine trap such as a TGF- β trap) can also promote natural killer cell-mediated killing of tumor cells. (i) TGF β RII trap fusion proteins or Fragments or a variant thereof.

[0152] In some embodiments, a fusion protein or a fragment or a variant thereof also comprises a cytokine trap and a PD-1 inhibitor or anti-PD-1 antibody or a fragment or a variant thereof. In some embodiments is a fusion protein or a fragment or a variant thereof comprising the cytokine trap (for instance, TGF- β trap) fused to a PD-1 inhibitor optionally via a cleavable or non-cleavable linker. In some embodiments, the cytokine trap (*e.g.*, TGF- β trap) is a cytokine receptor (*e.g.*, TGF β RII). In some embodiments, the cytokine receptor sequence in a fusion protein described herein comprises an extracellular domain (ECD) of the receptor (*e.g.*, TGF β RII) or a functional variant or derivative thereof. In some embodiments, the extracellular domain (ECD) of TGF β RII comprises a polypeptide sequence as shown in SEQ ID NO: 14. In some embodiments, the cytokine receptor sequence in a fusion protein or a fragment or a variant thereof described herein comprises a polypeptide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with the polypeptide sequence of SEQ ID NO: 14. In some embodiments, the cytokine receptor sequence in a fusion protein described herein comprises an extracellular domain (ECD) of the receptor (*e.g.*, TGF β RII) or a functional variant or derivative thereof. In some embodiments, the extracellular domain (ECD) of TGF β RII comprises a polypeptide sequence as shown in SEQ ID NO: 141. In some embodiments, the cytokine receptor sequence in a fusion protein or a fragment or a variant thereof described herein comprises a polypeptide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with the polypeptide sequence of SEQ ID NO: 141. In some embodiments, the cytokine receptor sequence in a fusion protein described herein comprises an extracellular domain (ECD) of the receptor (*e.g.*, TGF β RII) or a functional variant or derivative thereof. In some embodiments, the extracellular domain (ECD) of TGF β RII comprises a polypeptide sequence as shown in SEQ ID NO: 142. In some embodiments, the cytokine receptor sequence in a fusion protein or a fragment or a variant thereof described herein comprises a polypeptide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with the polypeptide sequence of SEQ ID NO: 142. In some embodiments, the cytokine receptor sequence in a fusion protein or a fragment or a variant thereof described herein binds TGF- β 1 and/or TGF- β 3 but does not bind TGF- β 2. In certain embodiments, the cytokine receptor sequence in a fusion protein or a fragment or a variant thereof described herein only binds TGF- β 1. In

certain embodiments, the cytokine receptor sequence in a fusion protein or a fragment or a variant thereof described herein only binds TGF- β 3. In certain embodiments, the cytokine receptor sequence in a fusion protein or a fragment or a variant thereof described herein only binds TGF- β 1 and/or TGF- β 3 but has low to no affinity for TGF- β 2.

- [0153] In some embodiments, a PD-1 antibody is fused to TGF β RII or a fragment thereof (*e.g.*, ECD of TGF β RII). In some embodiments, a PD-1 antibody moiety is fused to TGF β RII or a fragment thereof (*e.g.*, ECD of TGF β RII) via a linker. In some embodiments, a PD-1 antibody moiety is fused to at least one extracellular domain of TGF β RII. In some embodiments, a PD-1 antibody moiety is fused to at least one extracellular domain of TGF β RII via a linker.
- [0154] In some embodiments, a PD-1 antibody fragment or variant is a Fab, Fab₂, (Fab')₂, Fv, (Fv)₂, scFv, scFv-F_C, F_C, diabody, triabody, or minibody of the PD-1 antibody. In some embodiments, a PD-1 antibody fragment is a single-domain antibody of the PD-1 antibody. In some embodiments, the single-domain antibody is a V_{NAR} or V_HH fragment of the PD-1 antibody.
- [0155] Non-limiting exemplary fusion proteins are illustrated in FIGS. 4A-4C. In some embodiments, the fusion protein comprising an anti-PD-1 antibody or a fragment or a variant thereof fused to a TGF- β trap can elicit a synergistic anti-tumor effect due to the simultaneous blockade of the interaction between PD-L1 on tumor cells and PD-1 on immune cells, and the neutralization of TGF- β in the tumor microenvironment. Without being bound by theory, this effect is obtained from simultaneous blocking the two major immune escape mechanisms and the targeted depletion of the TGF- β in the tumor microenvironment by a single molecular entity. This depletion is achieved by (1) PD-1 targeting of tumor cells; (2) binding of the TGF- β in the tumor microenvironment by the TGF- β trap (*e.g.*, TGF β RII); and (3) destruction of the bound TGF- β through the PD-L1 receptor mediated endocytosis.
- [0156] In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a variable region of heavy chain (V_H) of PD-1 antibody or a fragment/variant thereof. In other embodiments, the TGF- β trap is fused to IgG of a PD-1 antibody (for example, FIG. 4A). In certain aspects, the IgG is IgG1, IgG2, IgG3, or IgG4. In an embodiment, the IgG is IgG4. In another embodiment, the IgG4 is SEQ ID NO 146 (wild type), SEQ ID NO: 291, SEQ ID NO: 292 or SEQ ID NO: 147 (S108P). In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a variable region of heavy chain (V_H) of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a

constant region of the V_H of PD-1 antibody or a fragment/variant thereof via a linker.

Examples of V_H sequence of a PD-1 antibody or fragment or variant thereof include but are not limited to SEQ ID NOs.: 1-7 and 149-164. Examples of V_L sequence of a PD-1 antibody or fragment or variant thereof include but are not limited to SEQ ID NOs.: 8-13 and 148. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused a variable region of light chain (V_L) of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a constant region of the V_L of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused a variable region of light chain (V_L) of PD-1 antibody or a fragment/variant thereof via a linker. In one aspect, the TGF- β trap (*e.g.*, TGF β RII) is fused to either the N- or C-terminus of the V_L or V_H chain or a fragment/variant thereof via a linker.

[0157] The term “anti-PD1 (VL/VH)-TGF β RII” or “anti-PD1 (VH/VL)-TGF β RII” is used interchangeably and denotes the specific V_L or V_H used in the fusion protein. In one embodiment, the terms “anti-PD1 (VL/VH)-TGF β RII” or “anti-PD1 (VH/VL)-TGF β RII” refers to TGF- β trap (*e.g.*, TGF β RII) fused to constant region of the heavy chain of anti-PD1 or alternatively, refers to TGF- β trap (*e.g.*, TGF β RII) fused to constant region of the light chain of anti-PD1.

[0158] In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a Fab of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a Fab of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a Fab₂ of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a Fab₂ of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a (Fab')₂ of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a (Fab')₂ of PD-1 antibody or a fragment/variant thereof via a linker. In one aspect, the TGF- β trap (*e.g.*, TGF β RII) is fused to either the N- or C-terminus of the Fab or (Fab')₂ of PD-1 antibody or a fragment/variant thereof via a linker.

[0159] In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a Fv of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a Fv of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a (Fv)₂ of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a (Fv)₂ of PD-1 antibody or a fragment/variant thereof via a linker. In one aspect, the TGF- β

trap (*e.g.*, TGF β RII) is fused to either the N- or C-terminus of the Fv or (Fv)₂ of PD-1 antibody or a fragment/variant thereof via a linker.

- [0160] In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a scFv of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a scFv of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a scFv-F_C of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a scFv-F_C of PD-1 antibody or a fragment/variant thereof via a linker. In one aspect, the TGF- β trap (*e.g.*, TGF β RII) is fused to either the N- or C-terminus of the scFv or scFv-F_C of PD-1 antibody or a fragment/variant thereof via a linker.
- [0161] In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a F_C of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a F_C of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is linked to a C-terminus F_C of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is linked to a C-terminus F_C of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is linked to a N-terminus F_C of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is linked to a N-terminus F_C of PD-1 antibody or a fragment/variant thereof via a linker.
- [0162] In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a diabody of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a diabody of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a triabody of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a triabody of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a minibody of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a minibody of PD-1 antibody or a fragment/variant thereof via a linker. In one aspect, the TGF- β trap (*e.g.*, TGF β RII) is fused to either the N- or C-terminus of the minibody of PD-1 antibody or a fragment/variant thereof via a linker.
- [0163] In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a V_{NAR} of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a V_{NAR} of PD-1 antibody or a fragment/variant thereof via a linker. In

all embodiments, as described, the TGF- β trap (*e.g.*, TGF β RII) is fused to either the N- or C-terminus of the V_{NAR} of PD-1 antibody or a fragment/variant thereof via a linker.

[0164] In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a V_HH of PD-1 antibody or a fragment/variant thereof. In some embodiments, the TGF- β trap (*e.g.*, TGF β RII) is fused to a V_HH of PD-1 antibody or a fragment/variant thereof via a linker. In all embodiments, as described, the TGF- β trap (*e.g.*, TGF β RII) is fused to either the N- or C-terminus of the V_HH of PD-1 antibody or a fragment/variant thereof via a linker.

[0165] In some embodiments, a PD-1 antibody moiety is fused to TGF β RII or a fragment or a variant thereof (*e.g.*, ECD of TGF β RII). In some embodiments, a PD-1 antibody moiety is fused to TGF β RII or a fragment or a variant thereof (*e.g.*, ECD of TGF β RII) via a linker. In some embodiments, a PD-1 antibody moiety is fused to at least one extracellular domain of TGF β RII. In some embodiments, a PD-1 antibody moiety is fused to at least one extracellular domain of TGF β RII via a linker. Non-limiting exemplary fusion proteins are illustrated in FIGs. 4A-4C.

[0166] In some embodiments, a PD-1 antibody fragment is a Fab, Fab₂, (Fab')₂, Fv, (Fv)₂, scFv, scFv-F_C, F_C, diabody, triabody, or minibody of the PD-1 antibody. In some embodiments, a PD-1 antibody fragment is a single-domain antibody of the PD-1 antibody. In some embodiments, the single-domain antibody is a V_{NAR} or V_HH fragment of the PD-1 antibody.

[0167] In some embodiments, the variable region of heavy chain (V_H) of PD-1 antibody or a fragment/variant thereof comprises one or more polypeptide sequences as shown in any one of SEQ ID NOs: 1-7. In some embodiments, the variable region of heavy chain (V_H) of PD-1 antibody or a fragment/variant thereof comprises one or more polypeptide sequences having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with any one of polypeptide sequences as shown in SEQ ID NOs: 1-7 and 149-164.

[0168] In some embodiments, the variable region of heavy chain (V_L) of PD-1 antibody or a fragment/variant thereof comprises one or more polypeptide sequences as shown in any one of SEQ ID NOs: 8-13 and 148. In some embodiments, the variable region of heavy chain (V_L) of PD-1 antibody or a fragment/variant thereof comprises one or more polypeptide sequences having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with any one of polypeptide sequences as shown in SEQ ID NOs: 8-13 and 148.

- [0169] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 6 and a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 12. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 6 and a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 12.
- [0170] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 15 and a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 16.
- [0171] In some embodiments, the fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 16.
- [0172] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 15 and a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 143.
- [0173] In some embodiments, the fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 143.
- [0174] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 15 and a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 294.
- [0175] In embodiments, the fusion protein comprises a sequence as shown in SEQ ID NO: 15 and a sequence as shown in SEQ ID NO: 294.

- [0176] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 7 and a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 13. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 7 and a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 13.
- [0177] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 296 and a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 145.
- [0178] In some embodiments, the fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 145.
- [0179] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 296 and a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 144.
- [0180] In some embodiments, the fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 144.
- [0181] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 296 and a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 295.

- [0182] In some embodiments, the fusion protein comprises a sequence as shown in SEQ ID NO: 296 and a sequence as shown in SEQ ID NO: 295.
- [0183] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 12 and a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 16.
- [0184] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 12 and a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 143.
- [0185] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 13 and a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 145. In some embodiments, the fusion protein comprises a sequence as shown in SEQ ID NO: 145.
- [0186] In some embodiments, the fusion protein comprises a linker and a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 16 and a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 15. In some embodiments, the fusion protein comprises a linker and a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 16 and a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 15.
- [0187] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 1. In some embodiments,

the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 1.

[0188] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 2. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 2.

[0189] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 3. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 3.

[0190] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 4. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 4.

[0191] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 5. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 5.

[0192] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 6. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 6.

- [0193] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 7. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 7.
- [0194] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 5 and a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 8. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 5 and a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 8.
- [0195] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 149 and a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 8. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 149 and a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 8.
- [0196] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 157 and a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 8. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 157 and a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%,

at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 8.

[0197] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 158 and a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 8. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 158 and a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 8.

[0198] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 5. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 5.

[0199] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 149. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 149.

[0200] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 157. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 157.

[0201] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 158. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%,

at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 158.

[0202] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 158. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 158.

[0203] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 8. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 8.

[0204] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 149. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 149.

[0205] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 150. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 150.

[0206] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 151. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 151.

[0207] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 152. In some

embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 152.

[0208] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 153. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 153.

[0209] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 154. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 154.

[0210] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 155. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 155.

[0211] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 156. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 156.

[0212] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 157. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 157.

- [0213] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 158. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 158.
- [0214] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 159. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 159.
- [0215] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 160. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 160.
- [0216] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 161. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 161.
- [0217] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 162. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 162.
- [0218] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 163. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%,

at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 163.

[0219] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 164. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 164.

[0220] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 8. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 8.

[0221] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 9. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 9.

[0222] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 10. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 10.

[0223] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 11. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 11.

[0224] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 12. In some

embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 12.

[0225] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 13. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 13.

[0226] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 148. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 148.

[0227] In some embodiments, the fusion protein comprises a polypeptide sequence as shown in SEQ ID NO: 15. In some embodiments, the fusion protein comprises a polypeptide sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 15.

[0228] In some embodiments, the fusion protein comprises a polypeptide sequence as shown in SEQ ID NO: 16. In some embodiments, the fusion protein comprises a polypeptide sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 16.

[0229] In some embodiments, the fusion protein comprises a polypeptide sequence as shown in SEQ ID NO: 143. In some embodiments, the fusion protein comprises a polypeptide sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 143.

[0230] In some embodiments, the fusion protein comprises a polypeptide sequence as shown in SEQ ID NO: 144. In some embodiments, the fusion protein comprises a polypeptide sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least

70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 144.

[0231] In some embodiments, the fusion protein comprises a polypeptide sequence as shown in SEQ ID NO: 145. In some embodiments, the fusion protein comprises a polypeptide sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 145.

(ii) Anti-TGF β antibodies –fusion proteins or Fragments or Variant Thereof

[0232] In other embodiments, the cytokine trap is an antibody, antibody fragment or an antibody variant against TGF- β . In such embodiments, such antibodies can include pan-neutralizing anti-TGF β antibody, or an anti-receptor antibody that blocks the receptor from binding to TGF β 1, 2 and/or 3. In certain embodiments, the antibody fragment or variant is a Fab, Fab₂, (Fab')₂, Fv, (Fv)₂, scFv, scFv-F_C, F_C, diabody, triabody, or minibody of the TGF β antibody. In one embodiment, the anti- TGF- β antibody or fragment or variant thereof binds to TGF- β 1, TGF- β 2, and TGF- β 3. In certain embodiments, the anti- TGF- β antibody or fragment or variant thereof binds to TGF- β 1. In certain embodiments, the anti-TGF- β antibody or fragment or variant thereof binds to TGF- β 3. In certain embodiments, the anti- TGF- β antibody or fragment or variant thereof binds to TGF- β 1 and TGF- β 2. In certain embodiments, the anti- TGF- β antibody or fragment or variant thereof binds to TGF- β 1 and TGF- β 3. Examples of VH sequence of a TGF- β antibody or fragment or variant thereof include but are not limited to SEQ ID Nos. 166, 168, 169, 171, 173, 175, and 177. Examples of VL sequence of a TGF- β antibody or fragment or variant thereof include but are not limited to SEQ ID NOs: 165, 167, 170, 172, 174, 176, and 178. In some embodiments, the anti-TGF- β antibody comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence selected from any one of SEQ ID NOs: 166, 168, 169, 171, 173, 175, or 177. In some embodiments, the anti-TGF- β antibody comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence selected from any one of SEQ ID NOs: 165, 167, 170, 172, 174, 176, or 178.

(iii) TGF- β antagonistic peptides- fusion proteins or Fragments or Variant Thereof

[0233] In certain embodiments, the cytokine trap can include TGF- β antagonistic peptides or TGF- β inhibitory peptides. Such peptides can be generated de novo using phage display. In one embodiment, such peptides can be derived from segments of TGF- β isoform(s) or TGF- β receptor(s). Examples of TGF- β antagonistic peptides can include but are not limited to SEQ ID NO. 193-227. Examples of TGF- β inhibitory peptides can include but are not limited to SEQ ID NOs: SEQ ID NO. 193-227. In one embodiment, the peptides can be fused to V_H and/or V_L of a PD-1 antibody or a fragment/variant thereof via a linker. In another embodiment, one peptide can be fused to a PD-1 antibody or a fragment/variant thereof. In a further embodiment, more than one peptide can be fused to a PD-1 antibody or a fragment/variant thereof. When more than one peptide is utilized, the peptide can be fused with or without a linker to form concatemers. When more than one peptide is utilized, the same peptide can be used to form concatemers. Alternatively, a combination of two or more different peptides can be used to form concatemers.

Adenosine:

[0234] Adenosine is a key immune modulator in the tumor microenvironment. Extracellular adenosine suppresses inflammatory response upon binding to the A2A adenosine receptor (A2AR) which is predominantly expressed subtype in most immune cells. Several tumors express high levels of CD39 and CD73, the ectonucleotidases responsible for converting ATP and ADP to AMP, and AMP to adenosine, respectively. Accordingly, adenosine promotes suppressive activity of regulatory T cells by inducing the expression of Foxp3, CD39 and CD73. Additionally, hypoxia induces the accumulation of extracellular adenosine in the tumor microenvironment through the induction of CD39 and CD73. In addition, high levels of extracellular adenosine in the tumor microenvironment is maintained by the hypoxia-inducible factor(HIF)-dependent inhibition of the nucleotide transporter ENT-1, and the inhibition of adenosine kinase preventing the relocation of adenosine in the intracellular space and preventing the formation of AMP, respectively. Accordingly, targeting the reduction of extracellular adenosine in the tumor microenvironment is understood to enhance immune cell function and promote tumor cell killing.

[0235] In embodiments provided herein are fusion proteins that comprise an adenosine deaminase (*e.g.*, ADA2) to target reduction of extracellular adenosine in the tumor microenvironment.

Adenosine deaminase

[0236] Adenosine deaminase (also known as adenosine aminohydrolase, or ADA) ADA irreversibly deaminates adenosine, converting it to the related nucleoside inosine by the substitution of the amino group for a keto group. Inosine can then be deribosylated (removed from ribose) by another enzyme called purine nucleoside phosphorylase (PNP), converting it to hypoxanthine. ADA is needed for the breakdown of adenosine from food and for the turnover of nucleic acids in tissues. Its primary function in humans is the development and maintenance of the immune system. However, ADA association has also been observed with epithelial cell differentiation, neurotransmission, and gestation maintenance. There are 2 isoforms of ADA: ADA1 and ADA2.

[0237] ADA1 is found in most body cells, particularly lymphocytes and macrophages, where it is present not only in the cytosol and nucleus but also as the ecto- form on the cell membrane attached to dipeptidyl peptidase-4 (aka, CD26). ADA1 is involved mostly in intracellular activity, and exists both in small form (monomer) and large form (dimer). The interconversion of small to large forms is regulated by a 'conversion factor' in the lung.

[0238] ADA2 was first identified in human spleen. It was subsequently found in other tissues including the macrophage where it co-exists with ADA1. The two isoforms regulate the ratio of adenosine to deoxyadenosine. ADA2 is found predominantly in the human plasma and serum, and exists mainly as a homodimer. ADA2 is the predominant form present in human blood plasma and is increased in many diseases, particularly those associated with the immune system: for example rheumatoid arthritis, psoriasis, and sarcoidosis. The plasma ADA2 isoform is also increased in most cancers. ADA2 is not ubiquitous but co-exists with ADA1 in monocytes-macrophages.

ADA2 Fusion Proteins or Fragments or Variant Thereof

[0239] Provided herein is a fusion protein or a fragment or a variant thereof comprising an immune checkpoint inhibitor, such as a PD-1 inhibitor or antibody, and an adenosine deaminase (*e.g.*, ADA2) that can neutralize adenosine. The fusion protein or a fragment or a variant thereof provided herein (*e.g.*, PD-1 inhibitor or antibody fused to an adenosine deaminase (*e.g.*, ADA2) can elicit a synergistic anti-tumor effect due to the simultaneous blockade of the interaction between *e.g.*, PD-L1 on tumor cells and PD-1 on immune cells, and the neutralization of *e.g.*, adenosine in the tumor microenvironment. Without being bound by theory, this effect is obtained from simultaneous blocking the two major immune escape mechanisms and the targeted depletion of adenosine in the tumor microenvironment by a single molecular entity. This depletion can be achieved by one or more of the

following: (1) anti-PD-1 targeting of tumor cells; (2) binding of the adenosine in the tumor microenvironment by an adenosine deaminase (*e.g.*, ADA2); and (3) destruction of the bound adenosine through the PD-L1 receptor mediated endocytosis.

[0240] In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is a part of a fusion protein or a fragment or a variant thereof also comprising PD-1 inhibitor or antibody or a fragment or a variant thereof. In some embodiments is a fusion protein or a fragment or a variant thereof comprising the adenosine deaminase (*e.g.*, ADA2) fused to a PD-1 inhibitor optionally via a cleavable or non-cleavable linker. In some embodiments, an adenosine deaminase is an adenosine deaminase 2 (ADA2). In some embodiments, the fusion protein described herein comprises an adenosine deaminase (*e.g.*, ADA2) described herein or a functional variant or derivative thereof. Examples of ADA2 and variants are described in WO 2016061286, herein incorporated by reference in its entirety. In some embodiments, the TGF- β cytokine trap comprises any one of ADA2 mutant 1, ADA2 mutant 2, ADA2 mutant 3, ADA2 mutant 4, ADA2 mutant 5, ADA2 mutant 6, or ADA2 mutant 7. In some embodiments, the fusion protein or a fragment or a variant thereof provided herein comprises the PD-1 inhibitor or antibody fused to the ADA protein, or functional fragment thereof, via a linker. In some embodiments, the PD-1 inhibitor can be an antibody or a fragment or a variant of the antibody that targets PD-1.

[0241] In some embodiments, the fusion protein or a fragment or a variant thereof described herein comprises a polypeptide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with the polypeptide sequence of SEQ ID NO: 284. In some embodiments, the fusion protein or a fragment or a variant thereof described herein comprises a polypeptide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with the polypeptide sequence of SEQ ID NO: 273. In some embodiments, the fusion protein or a fragment or a variant thereof described herein comprises a polypeptide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with the polypeptide sequence of SEQ ID NO: 274. In some embodiments, the fusion protein or a fragment or a variant thereof described herein comprises a polypeptide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with the polypeptide sequence of SEQ ID NO:

37275. In some embodiments, the fusion protein or a fragment or a variant thereof described herein comprises a polypeptide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with the polypeptide sequence of SEQ ID NO: 276. In some embodiments, the fusion protein or a fragment or a variant thereof described herein comprises a polypeptide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with the polypeptide sequence of SEQ ID NO: 277. In some embodiments, the fusion protein or a fragment or a variant thereof described herein comprises a polypeptide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with the polypeptide sequence of SEQ ID NO: 278. In some embodiments, the fusion protein or a fragment or a variant thereof described herein comprises a polypeptide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with the polypeptide sequence of SEQ ID NO: 279.

[0242] In some embodiments, the fusion protein or a fragment or a variant thereof described herein comprises an adenosine deaminase (*e.g.*, ADA2) as described above and an antibody, a fragment or a variant of the antibody that targets immune checkpoint genes. In some embodiments, an antibody or a fragment or a variant of the antibody that target immune checkpoints, such as cytotoxic T lymphocyte associated protein-4 (CTLA-4) and programmed cell death-1 (PD-1), can be fused to an adenosine deaminase (*e.g.*, ADA2) molecule via a linker.

[0243] In some embodiments, a PD-1 antibody moiety is fused to an adenosine deaminase (*e.g.*, ADA2) or a fragment thereof. In some embodiments, a PD-1 antibody moiety is fused to an adenosine deaminase (*e.g.*, ADA2) via a linker. In some embodiments, a PD-1 antibody moiety is fused to at least one domain of ADA2. In some embodiments, a PD-1 antibody moiety is fused to at least one domain of ADA2 via a linker.

[0244] In some embodiments, a PD-1 antibody fragment or variant is a Fab, Fab₂, (Fab')₂, Fv, (Fv)₂, scFv, scFv-F_C, F_C, diabody, triabody, or minibody of the PD-1 antibody. In some embodiments, a PD-1 antibody fragment is a single-domain antibody of the PD-1 antibody. In some embodiments, the single-domain antibody is a V_{NAR} or V_HH fragment of the PD-1 antibody.

- [0245] Non-limiting exemplary fusion proteins are illustrated in FIGs. 33A-33C. In some embodiments, the fusion protein comprising an anti-PD-1 antibody or a fragment or a variant thereof fused to an adenosine deaminase (*e.g.*, ADA2) can elicit a synergistic anti-tumor effect due to the simultaneous blockade of the interaction between PD-L1 on tumor cells and PD-1 on immune cells, and targeting of the reduction of extracellular adenosine in the tumor microenvironment.
- [0246] In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a variable region of heavy chain (V_H) of PD-1 antibody or a fragment/variant thereof. In other embodiments, the adenosine deaminase is fused to IgG of a PD-1 antibody (for example, Fig. 4a). In certain aspects, the IgG is IgG1, IgG2, IgG3, or IgG4. In an embodiment, the IgG is IgG4. In another embodiment, the IgG4 is SEQ ID NO 146 (wild type), SEQ ID NO: 291, SEQ ID NO: 292 or SEQ ID NO: 147 (S108P). In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a variable region of heavy chain (V_H) of PD-1 antibody or a fragment/variant thereof via a linker. . In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a constant region of the V_H of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused a variable region of light chain (V_L) of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused a variable region of light chain (V_L) of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a constant region of the V_L of PD-1 antibody or a fragment/variant thereof via a linker. In one aspect, the adenosine deaminase is fused to either the N- or C-terminus of the V_H or V_L of PD-1 antibody or a fragment/variant thereof via a linker.
- [0247] In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a Fab of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a Fab of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a Fab₂ of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a Fab₂ of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a (Fab')₂ of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a (Fab')₂ of PD-1 antibody or a fragment/variant thereof via a linker. In one aspect, the adenosine deaminase is fused to either the N- or C-terminus of the Fab or Fab₂ of PD-1 antibody or a fragment/variant thereof via a linker.

- [0248] In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a Fv of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a Fv of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a (Fv)₂ of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a (Fv)₂ of PD-1 antibody or a fragment/variant thereof via a linker. In one aspect, the adenosine deaminase is fused to either the N- or C-terminus of the Fv or (Fv)₂ of PD-1 antibody or a fragment/variant thereof via a linker.
- [0249] In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a scFv of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a scFv of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a scFv-F_C of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a scFv-F_C of PD-1 antibody or a fragment/variant thereof via a linker. In one aspect, the adenosine deaminase is fused to either the N- or C-terminus of the scFv or scFv-F_C of PD-1 antibody or a fragment/variant thereof via a linker.
- [0250] In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a F_C of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a F_C of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is linked to a C-terminus F_C of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is linked to a C-terminus F_C of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is linked to a N-terminus F_C of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is linked to a N-terminus F_C of PD-1 antibody or a fragment/variant thereof via a linker.
- [0251] In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a diabody of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a diabody of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a triabody of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a triabody of PD-1 antibody or a fragment/variant thereof via a linker. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a minibody of PD-1 antibody or a fragment/variant thereof. In some

embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a minibody of PD-1 antibody or a fragment/variant thereof via a linker.

[0252] In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a V_{NAR} of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a V_{NAR} of PD-1 antibody or a fragment/variant thereof via a linker. In one aspect, the adenosine deaminase is fused to either the N- or C-terminus of the V_{NAR} of PD-1 antibody or a fragment/variant thereof via a linker.

[0253] In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a V_{HH} of PD-1 antibody or a fragment/variant thereof. In some embodiments, the adenosine deaminase (*e.g.*, ADA2) is fused to a V_{HH} of PD-1 antibody or a fragment/variant thereof via a linker. In one aspect, the adenosine deaminase is fused to either the N- or C-terminus of the V_{HH} of PD-1 antibody or a fragment/variant thereof via a linker.

[0254] In some embodiments, the variable region of heavy chain (V_{H}) of PD-1 antibody or a fragment/variant thereof comprises one or more polypeptide sequences as shown in any one of SEQ ID NOs: 1-7. In some embodiments, the variable region of heavy chain (V_{H}) of PD-1 antibody or a fragment/variant thereof comprises one or more polypeptide sequences having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with any one of polypeptide sequences as shown in SEQ ID NOs: 1-7 and 149-164.

[0255] In some embodiments, the variable region of heavy chain (V_{L}) of PD-1 antibody or a fragment/variant thereof comprises one or more polypeptide sequences as shown in any one of SEQ ID NOs: 8-13 and 148. In some embodiments, the variable region of heavy chain (V_{L}) of PD-1 antibody or a fragment/variant thereof comprises one or more polypeptide sequences having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5% or 100% identity with any one of polypeptide sequences as shown in SEQ ID NOs: 8-13 and 148.

[0256] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_{H}) comprising a polypeptide sequence as shown in SEQ ID NO: 6 and a variable region of light chain (V_{L}) comprising a polypeptide sequence as shown in SEQ ID NO: 12. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_{H}) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 6 and a variable region of light chain (V_{L}) that is at least

50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 12.

[0257] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 12 and a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 280.

[0258] In some embodiments, the fusion protein comprises a sequence as shown in SEQ ID NO: 12 and a sequence as shown in SEQ ID NO: 280.

[0259] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 12 and a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 281.

[0260] In some embodiments, the fusion protein comprises a sequence as shown in SEQ ID NO: 12 and a sequence as shown in SEQ ID NO: 281.

[0261] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 7 and a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 13. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 7 and a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 13.

[0262] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 13 and a sequence that is at least 50%, at least 55%, at least 60%, at

least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 282.

[0263] In some embodiments, the fusion protein comprises a sequence as shown in SEQ ID NO: 13 and a sequence as shown in SEQ ID NO: 282.

[0264] In some embodiments, the fusion protein comprises a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 13 and a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 283.

[0265] In some embodiments, the fusion protein comprises a sequence as shown in SEQ ID NO: 13 and a sequence as shown in SEQ ID NO: 283.

[0266] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 1. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 1.

[0267] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 2. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 2.

[0268] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 3. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 3.

[0269] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 4. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%,

at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 4.

[0270] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 5. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 5.

[0271] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 6. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 6.

[0272] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 7. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 7.

[0273] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 149. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 149.

[0274] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 150. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 150.

[0275] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 151. In some

embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 151.

[0276] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 152. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 152.

[0277] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 153. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 153.

[0278] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 154. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 154.

[0279] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 155. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 155.

[0280] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 156. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 156.

- [0281]** In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 157. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 157.
- [0282]** In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 158. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 158.
- [0283]** In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 159. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 159.
- [0284]** In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 160. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 160.
- [0285]** In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 161. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 161.
- [0286]** In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 162. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%,

at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 162.

[0287] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 163. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 163.

[0288] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 164. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 164.

[0289] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 8. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 8.

[0290] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 9. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 9.

[0291] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 10. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 10.

[0292] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 11. In some

embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 11.

[0293] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 12. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 12.

[0294] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 13. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 13.

[0295] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 148. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 148.

[0296] In some embodiments, the fusion protein comprises a polypeptide sequence as shown in SEQ ID NO: 15. In some embodiments, the fusion protein comprises a polypeptide sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 15.

[0297] In some embodiments, the fusion protein comprises a polypeptide sequence as shown in SEQ ID NO: 280. In some embodiments, the fusion protein comprises a polypeptide sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 280.

[0298] In some embodiments, the fusion protein comprises a polypeptide sequence as shown in SEQ ID NO: 281. In some embodiments, the fusion protein comprises a

polypeptide sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 281.

[0299] In some embodiments, the fusion protein comprises a polypeptide sequence as shown in SEQ ID NO: 282. In some embodiments, the fusion protein comprises a polypeptide sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 282.

[0300] In some embodiments, the fusion protein comprises a polypeptide sequence as shown in SEQ ID NO: 283. In some embodiments, the fusion protein comprises a polypeptide sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 283.

[0301] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 5 and a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 8. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 5 and a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 8.

[0302] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 149 and a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 8. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 149 and a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 8.

- [0303]** In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 157 and a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 8. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 157 and a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 8.
- [0304]** In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 158 and a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 8. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 158 and a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 8.
- [0305]** In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 5. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 5.
- [0306]** In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 149. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 149.
- [0307]** In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 157. In some

embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 157.

[0308] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 158. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 158.

[0309] In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) comprising a polypeptide sequence as shown in SEQ ID NO: 158. In some embodiments, the fusion protein comprises a variable region of heavy chain (V_H) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 158.

[0310] In some embodiments, the fusion protein comprises a variable region of light chain (V_L) comprising a polypeptide sequence as shown in SEQ ID NO: 8. In some embodiments, the fusion protein comprises a variable region of light chain (V_L) that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.5%, or 100% identical to a sequence as shown in SEQ ID NO: 8.

Linkers

[0311] In some embodiments, the linker comprises one or more polypeptide sequences as shown in any one of SEQ ID NOs: 17-34. In some embodiments, the linker can be a flexible linker. Flexible linkers can be applied when a joined domain requires a certain degree of movement or interaction. Flexible linkers can be composed of small, non-polar (*e.g.*, Gly) or polar (*e.g.*, Ser or Thr) amino acids. A flexible linker can have sequences consisting primarily of stretches of Gly and Ser residues ("GS" linker). A non-limiting example of a flexible linker can have the sequence of (Gly-Gly-Gly-Gly-Ser) n , wherein n can be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. By adjusting the copy number " n ", the length of this exemplary GS linker can be optimized to achieve appropriate separation of functional domains, or to maintain necessary inter-domain interactions. Besides GS linkers, other flexible linkers can be utilized for recombinant fusion proteins. In some embodiment, a flexible linker can have

the sequence of (Gly)*n*, wherein *n* can be 6, 7, or 8. In some cases, flexible linkers can also be rich in small or polar amino acids such as Gly and Ser, but can contain additional amino acids such as Thr and Ala to maintain flexibility. In some cases, the linker described herein can be a rigid linker. A rigid linker can be utilized to maintain a fixed distance between domains of the fusion protein or a fragment or a variant thereof described herein. Non-limiting examples of rigid linkers can be: alpha helix-forming linkers, pro-rich sequence, (XP)*n*, X-Pro backbone, (EAAAK)*n* (*n* = 1-6). Rigid linkers can exhibit relatively stiff structures by adopting α -helical structures or by containing multiple Pro residues in some cases. In some embodiments, the immune checkpoint inhibitor, such as a PD-1 inhibitor, and the cytokine trap (*e.g.*, TGF- β trap) that can neutralize the cytokine (*e.g.*, TGF- β) in a fusion protein or a fragment or a variant thereof described herein can be separated by an intervening sequence encoding an intervening linker polypeptide. In some embodiments, the immune checkpoint inhibitor, such as a PD-1 inhibitor, and the ADA2 (or mutants thereof) in a fusion protein or a fragment or a variant thereof described herein can be separated by an intervening sequence encoding an intervening linker polypeptide. In certain embodiments, the linker polypeptide comprises disclosed in the table below:

Table 1. Linker amino acid sequences and polynucleotide sequences

SEQ ID NO	Linkers
17	DPGGGGSGGGGSNP GS
18	GGGGSGGGGSGSDPGS
19	DPGSGGGGSGGGGSGS
20	GGGGSGGGGSGGGGSDPGS
21	DPGSGGGGSGGGGSGGGGS
22	DPGSGSVPLGSGSNP GS
23	DPGSGGSVPLGSGGSNP GS
24	DPGVLEREDKPTTSKPNP GS
25	DPGVLEREDVPTTSYPNP GS
26	DPGVLEREDKVTTSKYNP GS
27	DPVLEREDKVTTSKNP GS
28	DIEGRMD
29	GEGKSSGSGSESKAS
30	GSTSGSGKPGSGEGSTKG
31	A(EAAAK) ₄ ALEA(EAAAK) ₄ A

32	(G4S) _n , n=1-10
33	(Gly) _n , n=6-8
34	(EAAAK) _n , n=1-6

[0312] In some embodiments, the linker can be a flexible linker, a rigid linker, an *in vivo* cleavable linker, or any combination thereof. In some cases, the linker can connect functional domains together (as in flexible and rigid linkers) or releasing free functional domain *in vivo* as in *in vivo* cleavable linkers. In some embodiments, linkers can improve biological activity, increase expression yield, and achieving desirable pharmacokinetic profiles. In some embodiments, the linker can also comprise hydrazone, peptide, disulfide, or thioester.

[0313] In some cases, the linker sequence described herein can include a flexible linker. Flexible linkers can be applied when a joined domain requires a certain degree of movement or interaction. Flexible linkers can be composed of small, non-polar (*e.g.*, Gly) or polar (*e.g.*, Ser or Thr) amino acids. A flexible linker can have sequences consisting primarily of stretches of Gly and Ser residues (“GS” linker). An example of a flexible linker can have the sequence of (G4S)_n, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. In some embodiment, a flexible linker can have the sequence of (Gly)_n, wherein n can be 6, 7, or 8. In some cases, flexible linkers can also be rich in small or polar amino acids such as Gly and Ser, but can contain additional amino acids such as Thr and Ala to maintain flexibility. In other cases, polar amino acids such as Lys and Glu can be used to improve solubility. By adjusting the copy number “n”, the length of these non-limiting exemplary linkers can be optimized to achieve appropriate separation of functional domains, or to maintain necessary inter-domain interactions. Besides GS linkers, other flexible linkers can be utilized for the fusion protein or a fragment or a variant thereof described herein. In some cases, flexible linkers can also be rich in small or polar amino acids such as Gly and Ser, but can contain additional amino acids such as Thr and Ala to maintain flexibility. In other cases, polar amino acids such as Lys and Glu can be used to improve solubility.

[0314] Flexible linkers included in linker sequences described herein, can be rich in small or polar amino acids such as Gly and Ser to provide good flexibility and solubility. Flexible linkers can be suitable choices when certain movements or interactions are desired for fusion protein or a fragment or a variant thereof domains. In addition, although flexible linkers do not have rigid structures, they can serve as a passive linker to keep a distance between functional domains. The length of flexible linkers can be adjusted to allow for proper

folding or to achieve optimal biological activity of the fusion protein or a fragment or a variant thereof.

[0315] The linker described herein can further include a rigid linker in some cases. A rigid linker can be utilized to maintain a fixed distance between domains of the fusion protein or a fragment or a variant thereof described herein. Examples of rigid linkers can be: alpha helix-forming linkers, pro-rich sequence, (XP)_n, X-Pro backbone, (EAAAK)_n (n = 1-6), to name a few. Rigid linkers can exhibit relatively stiff structures by adopting α -helical structures or by containing multiple Pro residues in some cases.

[0316] In some embodiments, the linker described herein can be a cleavable linker. In other cases a linker is not cleavable. Linkers that are not cleavable can covalently join functional domains of the fusion protein or a fragment or a variant thereof together to act as one molecule throughout an *in vivo* processes or an *ex vivo* process. A linker can also be cleavable *in vivo*. A cleavable linker can be introduced to release free functional domains *in vivo*. A cleavable linker can be cleaved by the presence of reducing reagents, proteases, to name a few. For example, a reduction of a disulfide bond can be utilized to produce a cleavable linker. In the case of a disulfide linker, a cleavage event through disulfide exchange with a thiol, such as glutathione, could produce a cleavage. In other cases, an *in vivo* cleavage of a linker in a recombinant fusion protein can also be carried out by proteases that can be expressed *in vivo* under pathological conditions (*e.g.* cancer or inflammation), in specific cells or tissues, or constrained within certain cellular compartments. In some cases, a cleavable linker can allow for targeted cleavage. For example, the specificity of many proteases can offer slower cleavage of a linker in constrained compartments. A cleavable linker can also comprise hydrazone, peptides, disulfide, or thioether. For example, a hydrazone can confer serum stability. In other cases, a hydrazone can allow for cleavage in an acidic compartment. An acidic compartment can have a pH up to 7. A linker can also include a thioether. A thioether can be nonreducible. A thioether can be designed for intracellular proteolytic degradation.

[0317] In some cases, a linker can be engineered linker. For example, a linker can be designed to comprise chemical characteristics such as hydrophobicity. In some cases, at least two different linker polypeptide sequences can encode the same polypeptide linker sequence. Methods of designing linkers can be computational. In some cases, computational methods can include graphic techniques. Computation methods can be used to search for suitable peptides from libraries of three-dimensional peptide structures derived from databases. For example, a Brookhaven Protein Data Bank (PDB) can be used to span the distance in space

between selected amino acids of a linker. In some cases, a polypeptide linker can also include one or more GS linker sequences, for instance, (G4S)_n, (GS)_n, (SG)_n, (GSG)_n, and (SGSG)_n, wherein n can be any number from zero to fifteen.

Methods of Treating Cancer

[0318] Also provided herein is a method of treating cancer with a fusion protein comprising an immune checkpoint inhibitor, such as a PD-1 inhibitor, and a cytokine trap (*e.g.*, TGF- β trap) that can neutralize the cytokine (*e.g.*, TGF- β). Also provided herein is a method of treating cancer with a fusion protein comprising an immune checkpoint inhibitor, such as a PD-1 inhibitor, and adenosine deaminase protein. The development of monoclonal antibodies targeting blockade of an immune checkpoint pathway, such as PD1 and PD-L1/2 signaling pathway and CTLA-4 and CD80/86 signaling pathway, have revolutionized cancer treatment demonstrating durable clinical activity in several cancer indications including, but not limited to, melanoma, non-small cell lung cancer, renal cell carcinoma, bladder cancer, head and neck squamous cell carcinoma, MSI-high colorectal carcinoma, Merkel cell carcinoma, and Hodgkin lymphoma. Despite the durable responses, the response rate remains very low, and several patients have developed resistance leading to disease progression. Furthermore, in several indications, such as ovarian, gastroesophageal, prostate, pancreas and many other cancers, checkpoint inhibitors have failed to show any substantive clinical responses.

[0319] The failure of checkpoint blockade can be attributed to the complexity of the immunosuppressive factors present in the tumor microenvironment. These factors can include, but not limited to, suppressive cells, such as myeloid derived suppressive cells, tumor-associated macrophages (TAM); suppressive cytokine and growth factors, such as TGF- β and interleukin 10 (IL-10); and metabolic derivative, such as adenosine and indoleamine 2,3-dioxygenase (IDO) byproducts. Anti-PD1-TGF β RII fusion protein provided herein is an example of a therapy that can target two negative suppressive pathways in the tumor microenvironment. These pathways can include cell intrinsic interactions mediated by tumor cells to immune, in which PD-1/PD-L1 interaction can play a major role, and cell extrinsic interactions mediated by immunosuppressive cytokines, of which TGF- β can be a prominent member.

[0320] In some embodiments, the cancer is, but not limited to, glioblastoma, colorectal, gastric, cervical, ovarian, pancreatic, prostate, breast, and renal cancers. In addition, the fusion proteins as described herein can be amenable to indications, such as in non-small cell

cancer (NSCL) and melanoma, in which response rate of checkpoint blockades is lower and TGF- β is highly expressed.

[0321] Cancers include, but are not limited to, B cell cancer, *e.g.*, multiple myeloma, Waldenstrom's macroglobulinemia, the heavy chain diseases, such as, for example, alpha chain disease, gamma chain disease, and mu chain disease, benign monoclonal gammopathy, and immunocytic amyloidosis, melanomas, breast cancer, lung cancer, bronchus cancer, colorectal cancer, prostate cancer (*e.g.*, metastatic, hormone refractory prostate cancer), pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, and the like. Other non-limiting examples of types of cancers applicable to the methods encompassed by the present disclosure include human sarcomas and carcinomas, *e.g.*, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, colorectal cancer, pancreatic cancer, breast cancer, triple negative breast cancer, ovarian cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, liver cancer, hepatocellular carcinoma (HCC), choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, bone cancer, brain tumor, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, *e.g.*, acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease. In some embodiments, the cancer whose phenotype is determined by the method of the present disclosure is an epithelial cancer such as, but not limited to, bladder cancer, breast cancer,

cervical cancer, colon cancer, gynecologic cancers, renal cancer, laryngeal cancer, lung cancer, oral cancer, head and neck cancer, ovarian cancer, pancreatic cancer, prostate cancer, or skin cancer. In other embodiments, the cancer is breast cancer, prostate cancer, lung cancer, or colon cancer. In still other embodiments, the epithelial cancer is non-small-cell lung cancer, nonpapillary renal cell carcinoma, cervical carcinoma, ovarian carcinoma (*e.g.*, serous ovarian carcinoma), or breast carcinoma. The epithelial cancers can be characterized in various other ways including, but not limited to, serous, endometrioid, mucinous, clear cell, brenner, or undifferentiated. In some embodiments, the present disclosure is used in the treatment, diagnosis, and/or prognosis of lymphoma or its subtypes, including, but not limited to, mantle cell lymphoma.

[0322] In certain embodiments, anti-PD1-TGF β RII fusion protein provided herein is an example of a therapy that can be used to treat cancers that have about 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 or 95% average response rate to standard therapy (including but not limited to chemotherapy, chemotherapy and current clinical trial therapies). In certain embodiments, anti-PD1-ADA2 fusion protein provided herein is an example of a therapy that can be used to treat cancers that have about 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 or 95% average response rate to standard therapy (including but not limited to chemotherapy, chemotherapy and current clinical trial therapies). Such cancers include but are not limited to, Hodgkin's lymphoma, melanoma, non-small cell lung cancer (NSCLC), microsatellite instability (MSI)-high or mismatch repair (MMR)-deficient solid tumors, CSCC, RCC, CRC, melanoma, Merkel cell cancer, bladder cancer, RCC, hepatocellular carcinoma (HCC), head & neck cancer (H&N), cervical cancer, gastric cancer, small cell lung cancer (SCLC), endometrial cancer, mesothelioma, ovarian cancer, triple negative breast cancer (TNBC), breast cancer, colorectal cancer (CRC), pancreatic cancer, prostate cancer.

Combination Therapy

[0323] In some embodiments, the fusion protein is administered as a combination therapy with an additional therapeutic agent. In some embodiments, an additional therapeutic agent comprises a biological molecule, such as an antibody. For example, treatment can involve the combined administration of the fusion protein with antibodies against tumor-associated antigens including, but not limited to, antibodies that bind EGFR, HER2/ErbB2, and/or VEGF. In certain embodiments, the additional therapeutic agent is an antibody specific for a cancer stem cell marker. In certain embodiments, the additional therapeutic agent is an

antibody that is an angiogenesis inhibitor (*e.g.*, an anti-VEGF or VEGF receptor antibody). In certain embodiments, the additional therapeutic agent is bevacizumab (AVASTIN), ramucirumab, trastuzumab (HERCEPTIN), pertuzumab (OMNITARG), panitumumab (VECTIBIX), nimotuzumab, zalutumumab, or cetuximab (ERBITUX). In some embodiments, an additional therapeutic agent comprises an agent such as a small molecule. For example, treatment can involve the combined administration of the fusion protein or a fragment or a variant thereof provided herein with a small molecule that acts as an inhibitor against tumor-associated antigens including, but not limited to, EGFR, HER2 (ErbB2), and/or VEGF. In some embodiments, the fusion protein or a fragment or a variant thereof is administered in combination with a protein kinase inhibitor selected from the group consisting of: gefitinib (IRESSA), erlotinib (TARCEVA), sunitinib (SUTENT), lapatanib, vandetanib (ZACTIMA), AEE788, CI-1033, cediranib (RECENTIN), sorafenib (NEXAVAR), and pazopanib (GW786034B). In some embodiments, an additional therapeutic agent comprises an mTOR inhibitor. In another embodiment, the additional therapeutic agent is chemotherapy or other inhibitors that reduce the number of T_{REG} cells. In certain embodiments, the therapeutic agent is cyclophosphamide or an anti-CTLA4 antibody. In another embodiment, the additional therapeutic reduces the presence of myeloid-derived suppressor cells. In a further embodiment, the additional therapeutic is carbotaxol. In a further embodiment, the additional therapeutic agent is ibrutinib.

[0324] In some embodiments, the method can further comprise one or more checkpoint inhibitors in combination with the fusion protein or a fragment or a variant thereof as described herein. In some embodiments, the additional checkpoint inhibitor can be an anti-CTLA-4 antibody. The anti-CTLA-4 antibody (*e.g.*, ipilimumab) has shown durable anti-tumor activities and prolonged survival in participants with advanced melanoma, resulting in its Food and Drug Administration (FDA) approval in 2011. See Hodi *et al.*, Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med.* (2010) Aug 19; 363(8):711-23. In some embodiments, the one or more checkpoint inhibitors can be an anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 antibody can be a full length atezolizumab (anti-PD-L1), avelumab (anti-PD-L1), durvalumab (anti-PD-L1), or a fragment or a variant thereof. In some embodiments, the one or more checkpoint inhibitors can be any one or more of CD27 inhibitor, CD28 inhibitor, CD40 inhibitor, CD122 inhibitor, CD137 inhibitor, OX40 (also known as CD134) inhibitor, GITR inhibitor, ICOS inhibitor, or any combination thereof. In some embodiments, the one or more checkpoint inhibitors can be any one or more of A2AR inhibitor, B7-H3 (also known as CD276) inhibitor, B7-H4 (also

known as VTCN1) inhibitor, BTLA inhibitor, IDO inhibitor, KIR inhibitor, LAG3 inhibitor, TIM-3 inhibitor, VISTA inhibitor, or any combination thereof.

[0325] In certain embodiments, an additional therapeutic agent comprises a second immunotherapeutic agent. In some embodiments, the additional immunotherapeutic agent includes, but is not limited to, a colony stimulating factor, an interleukin, an antibody that blocks immunosuppressive functions (*e.g.*, an anti-CTLA-4 antibody, anti-CD28 antibody, anti-CD3 antibody, anti-PD-L1 antibody, anti-TIGIT antibody), an antibody that enhances immune cell functions (*e.g.*, an anti-GITR antibody, an anti-OX-40 antibody, an anti-CD40 antibody, or an anti-4-1BB antibody), a toll-like receptor (*e.g.*, TLR4, TLR7, TLR9), a soluble ligand (*e.g.*, GITRL, GITRL-Fc, OX-40L, OX-40L-Fc, CD40L, CD40L-Fc, 4-1BB ligand, or 4-1BB ligand-Fc), or a member of the B7 family (*e.g.*, CD80, CD86). In some embodiments, the additional immunotherapeutic agent targets CTLA-4, CD28, CD3, PD-L1, TIGIT, GITR, OX-40, CD-40, or 4-1BB.

[0326] In some embodiments, the additional therapeutic agent is an additional immune checkpoint inhibitor. In some embodiments, the additional immune checkpoint inhibitor is an anti-PD-L1 antibody, an anti-CTLA-4 antibody, an anti-CD28 antibody, an anti-TIGIT antibody, an anti-LAG3 antibody, an anti-TIM3 antibody, an anti-GITR antibody, an anti-4-1BB antibody, or an anti-OX-40 antibody. In some embodiments, the additional therapeutic agent is an anti-TIGIT antibody. In some embodiments, the additional therapeutic agent is an anti-PD-L1 antibody selected from the group consisting of: BMS935559 (MDX-1105), atezolizumab (MPDL3280A), durvalumab (MEDI4736), and avelumab (MSB0010718C). In some embodiments, the additional therapeutic agent is an anti-CTLA-4 antibody selected from the group consisting of: ipilimumab (YERVOY) and tremelimumab. In some embodiments, the additional therapeutic agent is an anti-LAG-3 antibody selected from the group consisting of: BMS-986016 and LAG525. In some embodiments, the additional therapeutic agent is an anti-OX-40 antibody selected from the group consisting of: MEDI6469, MEDI0562, and MOXR0916. In some embodiments, the additional therapeutic agent is an anti-4-1BB antibody selected from the group consisting of: PF-05082566. In some embodiments, the fusion protein or a fragment or a variant thereof can be administered in combination with a biologic molecule selected from the group consisting of: cytokines, adrenomedullin (AM), angiopoietin (Ang), BMPs, BDNF, EGF, erythropoietin (EPO), FGF, GDNF, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor (SCF), GDF9, HGF, HDGF, IGF, migration-stimulating factor, myostatin (GDF-8),

NGF, neurotrophins, PDGF, thrombopoietin, TGF- α , TGF- β , TNF- α , VEGF, PlGF, gamma-IFN, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, and IL-18.

Cytokines

- [0327] In some cases, the cytokine comprises at least one chemokine, interferon, interleukin, lymphokine, tumor necrosis factor, or variant or combination thereof. In some cases, the cytokine is an interleukin. In some cases the interleukin is at least one of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33 and functional variants and fragments thereof. In some embodiments, the cytokines can be membrane bound or secreted. In embodiments, the cytokine is soluble IL-15, soluble IL-15/IL-15R α complex (*e.g.*, ALT-803). In certain cases, the interleukin can comprise membrane bound IL-15 (mbIL-15) or a fusion of IL-15 and IL-15R α . In some embodiments, a mbIL-15 is a membrane-bound chimeric IL-15 which can be co-expressed with a modified immune effector cell described herein. In some embodiments, the mbIL-15 comprises a full-length IL-15 (*e.g.*, a native IL-15 polypeptide) or fragment or variant thereof, fused in frame with a full length IL-15R α , fragment or variant thereof. In some cases, the IL-15 is indirectly linked to the IL-15R α through a linker. In some instances, the mbIL-15 is as described in Hurton et al., “Tethered IL-15 augments antitumor activity and promotes a stem-cell memory subset in tumor-specific T cells,” PNAS 2016. In some cases, the cytokine is expressed in the same immune effector cell as the CAR.
- [0328] In some embodiments, the mbIL-15 is expressed with a cell tag such as HER1t, HER-1t-1, CD20t-1 or CD20 as described herein. The mbIL-15 can be expressed in-frame with HER1t, HER-1t-1, CD20t-1 or CD20.
- [0329] In some embodiments, the mbIL-15 can be under the control of an inducible promoter for gene transcription. In one aspect, the inducible promoter can be a gene switch ligand inducible promoter. In some cases, an inducible promoter can be a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch, such as RHEOSWITCH® gene switch.
- [0330] In another aspect, the interleukin can comprise IL-12. In some embodiments, the IL-12 is a single chain IL-12 (scIL-12), protease sensitive IL-12, destabilized IL-12, membrane bound IL-12, intercalated IL-12. In some instances, the IL-12 variants are as described in WO2015/095249, WO2016/048903, WO2017/062953, all of which is incorporated by reference in their entireties. In some embodiments, the cytokines described above can be under the control of an inducible promoter for gene transcription. In one aspect, the

inducible promoter can be a gene switch ligand inducible promoter. In some cases, an inducible promoter can be a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch, such as RHEOSWITCH® gene switch.

[0331] In some embodiments, the fusion protein as described herein can be under the control of an inducible promoter for gene transcription. In one aspect, the inducible promoter can be a gene switch ligand inducible promoter. In some cases, an inducible promoter can be a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch, such as RHEOSWITCH® gene switch.

Gene Switch

[0332] Provided herein are gene switch polypeptides, polynucleotides encoding ligand-inducible gene switch polypeptides, and methods and systems incorporating these polypeptides and/or polynucleotides. The term “gene switch” refers to the combination of a response element associated with a promoter, and for instance, an ecdysone receptor (EcR) based system which, in the presence of one or more ligands, modulates the expression of a gene into which the response element and promoter are incorporated. Tightly regulated inducible gene expression systems or gene switches are useful for various applications such as gene therapy, large scale production of proteins in cells, cell based high throughput screening assays, functional genomics and regulation of traits in transgenic plants and animals. Such inducible gene expression systems can include ligand inducible heterologous gene expression systems.

[0333] An early version of EcR-based gene switch used *Drosophila melanogaster* EcR (DmEcR) and *Mus musculus* RXR (MmRXR) polypeptides and showed that these receptors in the presence of steroid, ponasteroneA, transactivate reporter genes in mammalian cell lines and transgenic mice (Christopherson et al., 1992; No et al., 1996). Later, Suhr et al., 1998 showed that non-steroidal ecdysone agonist, tebufenozide, induced high level of transactivation of reporter genes in mammalian cells through *Bombyx mori* EcR (BmEcR) in the absence of exogenous heterodimer partner.

[0334] International Patent Applications No. PCT/US97/05330 (WO 97/38117) and PCT/US99/08381 (WO99/58155) disclose methods for modulating the expression of an exogenous gene in which a DNA construct comprising the exogenous gene and an ecdysone response element is activated by a second DNA construct comprising an ecdysone receptor that, in the presence of a ligand therefor, and optionally in the presence of a receptor capable of acting as a silent partner, binds to the ecdysone response element to induce gene

expression. In this example, the ecdysone receptor was isolated from *Drosophila melanogaster*. Typically, such systems require the presence of the silent partner, preferably retinoid X receptor (RXR), in order to provide optimum activation. In mammalian cells, insect ecdysone receptor (EcR) is capable of heterodimerizing with mammalian retinoid X receptor (RXR) and, thereby, be used to regulate expression of target genes or heterologous genes in a ligand dependent manner. International Patent Application No. PCT/US98/14215 (WO 99/02683) discloses that the ecdysone receptor isolated from the silk moth *Bombyx mori* is functional in mammalian systems without the need for an exogenous dimer partner.

[0335] U.S. Pat. No. 6,265,173 discloses that various members of the steroid/thyroid superfamily of receptors can combine with *Drosophila melanogaster* ultraspiracle receptor (USP) or fragments thereof comprising at least the dimerization domain of USP for use in a gene expression system. U.S. Pat. No. 5,880,333 discloses a *Drosophila melanogaster* EcR and ultraspiracle (USP) heterodimer system used in plants in which the transactivation domain and the DNA binding domain are positioned on two different hybrid proteins. In each of these cases, the transactivation domain and the DNA binding domain (either as native EcR as in International Patent Application No. PCT/US98/14215 or as modified EcR as in International Patent Application No. PCT/US97/05330) were incorporated into a single molecule and the other heterodimeric partners, either USP or RXR, were used in their native state.

[0336] International Patent Application No. PCT/US01/0905 discloses an ecdysone receptor-based inducible gene expression system in which the transactivation and DNA binding domains are separated from each other by placing them on two different proteins results in greatly reduced background activity in the absence of a ligand and significantly increased activity over background in the presence of a ligand. This two-hybrid system is a significantly improved inducible gene expression modulation system compared to the two systems disclosed in applications PCT/US97/05330 and PCT/US98/14215. The two-hybrid system is believed to exploit the ability of a pair of interacting proteins to bring the transcription activation domain into a more favorable position relative to the DNA binding domain such that when the DNA binding domain binds to the DNA binding site on the gene, the transactivation domain more effectively activates the promoter (see, for example, U.S. Pat. No. 5,283,173). The two-hybrid gene expression system comprises two gene expression cassettes; the first encoding a DNA binding domain fused to a nuclear receptor polypeptide, and the second encoding a transactivation domain fused to a different nuclear receptor polypeptide. In the presence of ligand, it is believed that a conformational change is induced

which promotes interaction of the antibody with the TGF- β cytokine trap, thereby resulting in dimerization of the DNA binding domain and the transactivation domain. Since the DNA binding and transactivation domains reside on two different molecules, the background activity in the absence of ligand is greatly reduced.

[0337] Certain modifications of the two-hybrid system could also provide improved sensitivity to non-steroidal ligands for example, diacylhydrazines, when compared to steroidal ligands for example, ponasterone A (“PonA”) or muristerone A (“MurA”). That is, when compared to steroids, the non-steroidal ligands provided higher gene transcription activity at a lower ligand concentration. Furthermore, the two-hybrid system avoids some side effects due to overexpression of RXR that can occur when unmodified RXR is used as a switching partner. In a preferred two-hybrid system, native DNA binding and transactivation domains of EcR or RXR are eliminated and as a result, these hybrid molecules have less chance of interacting with other steroid hormone receptors present in the cell, thereby resulting in reduced side effects.

[0338] The ecdysone receptor (EcR) is a member of the nuclear receptor superfamily and is classified into subfamily 1, group H (referred to herein as “Group H nuclear receptors”). The members of each group share 40-60% amino acid identity in the E (ligand binding) domain (Laudet et al., A Unified Nomenclature System for the Nuclear Receptor Subfamily, 1999; Cell 97: 161-163). In addition to the ecdysone receptor, other members of this nuclear receptor subfamily 1, group H include: ubiquitous receptor (UR), Orphan receptor 1 (OR-1), steroid hormone nuclear receptor 1 (NER-1), RXR interacting protein-15 (RIP-15), liver x receptor β (LXR β), steroid hormone receptor like protein (RLD-1), liver x receptor (LXR), liver x receptor α (LXR α), farnesoid x receptor (FXR), receptor interacting protein 14 (RIP-14), and farnesol receptor (HRR-1).

[0339] In some cases, an inducible promoter (“IP”) can be a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch, such as Intrexon Corporation’s RHEOSWITCH[®] gene switch. In some cases, a gene switch can be selected from ecdysone-based receptor components as described in, but without limitation to, any of the systems described in: PCT/US2001/009050 (WO 2001/070816); U.S. Pat. Nos. 7,091,038; 7,776,587; 7,807,417; 8,202,718; PCT/US2001/030608 (WO 2002/029075); U.S. Pat. Nos. 8,105,825; 8,168,426; PCT/US52002/005235 (WO 2002/066613); U.S. App. No. 10/468,200 (U.S. Pub. No. 20120167239); PCT/US2002/005706 (WO 2002/066614); U.S. Pat. Nos. 7,531,326; 8,236,556; 8,598,409; PCT/US2002/005090 (WO 2002/066612); U.S. Pat. No. 8,715,959 (U.S. Pub. No. 20060100416); PCT/US2002/005234 (WO 2003/027266);

U.S. Pat. Nos. 7,601,508; 7,829,676; 7,919,269; 8,030,067; PCT/U52002/005708 (WO 2002/066615); U.S. App. No. 10/468,192 (U.S. Pub. No. 20110212528); PCT/US2002/005026 (WO 2003/027289); U.S. Pat. Nos. 7,563,879; 8,021,878; 8,497,093; PCT/US2005/015089 (WO 2005/108617); U.S. Pat. No. 7,935,510; 8,076,454; PCT/U52008/011270 (WO 2009/045370); U.S. App. No. 12/241,018 (U.S. Pub. No. 20090136465); PCT/US2008/011563 (WO 2009/048560); U.S. App. No. 12/247,738 (U.S. Pub. No. 20090123441); PCT/US2009/005510 (WO 2010/042189); U.S. App. No. 13/123,129 (U.S. Pub. No. 20110268766); PCT/US2011/029682 (WO 2011/119773); U.S. App. No. 13/636,473 (U.S. Pub. No. 20130195800); PCT/US2012/027515 (WO 2012/122025); WO 2018/132494 (PCT/US2018/013196); and, U.S. Pat. No. 9,402,919; each of which is incorporated by reference in its entirety.

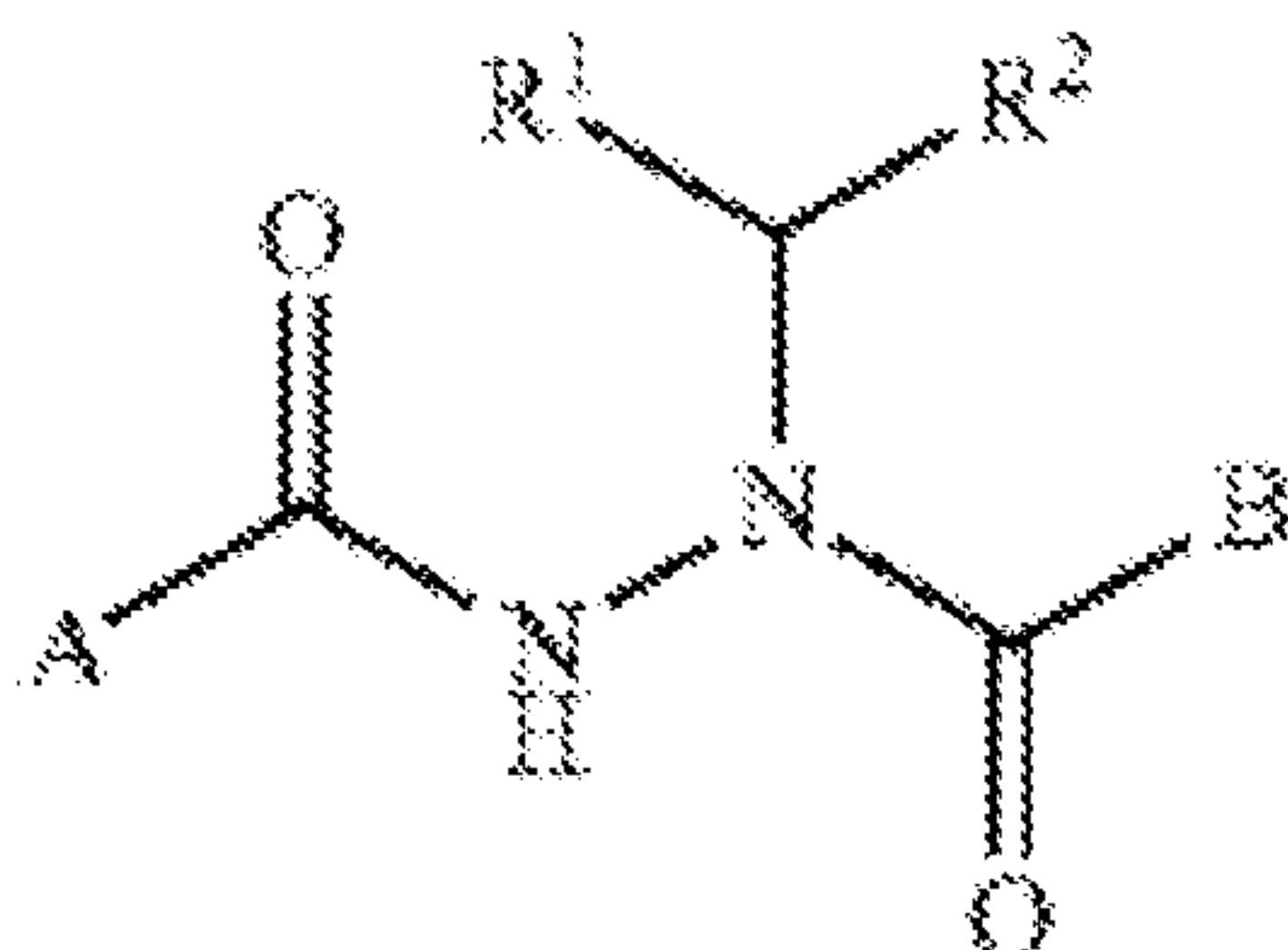
[0340] As used herein, the term “ligand,” as applied to ligand-activated ecdysone receptor-based gene switches are small molecules of varying solubility (such as diacylhydrazine compounds) having the capability of activating a gene switch to stimulate gene expression (*i.e.*, therein providing ligand inducible expression of polynucleotides (*e.g.*, mRNAs, miRNAs, *etc*) and/or polypeptides). Examples of such ligands include, but are not limited to those described in: WO 2004/072254 (PCT/US2004/003775); WO 2004/005478 (PCT/US2003/021149); WO 2005/017126 (PCT/US2004/005149); WO 2004/078924 (PCT/US2004/005912); WO 2008/153801 (PCT/US2008/006757); WO 2009/114201 (PCT/US2009/001639); WO 2013/036758 (PCT/US2012/054141); WO 2014/144380 (PCT/US2014/028768); and, WO 2016/044390 (PCT/US2015/050375); each of which are hereby incorporated by reference herein in the entirety.

[0341] Examples of ligands also include, without limitation, an ecdysteroid, such as ecdysone, 20-hydroxyecdysone, ponasterone A, muristerone A, and the like, 9-cis-retinoic acid, synthetic analogs of retinoic acid, N,N'-diacylhydrazines such as those disclosed in U.S. Pat. Nos.: 6,258,603; 6,013,836; 5,117,057; 5,530,028; 5,378,726; 7,304,161; 7,851,220; 8,748,125; 9,272,986; 7,456,315; 7,563,928; 8,524,948; 9,102,648; 9,169,210; 9,255,273; and, 9,359,289; oxadiazolines as described in U.S. Patent Nos.: 8,669,072; and, 8,895,306; dibenzoylalkyl cyanohydrazines such as those disclosed in European Application No. 2,461,809; N-alkyl-N,N'-diaroylhydrazines such as those disclosed in U.S. Pat. No. 5,225,443; N-acyl-N-alkylcarbonylhydrazines such as those disclosed in European Application No. 234,994; N-aroyl-N-alkyl-N'-aroylhydrazines such as those described in U.S. Pat. No. 4,985,461; amidoketones such as those described in U.S. Patent Nos.: 7,375,093; 8,129,355; and, 9,802,936; each of which is incorporated herein by reference and

other similar materials including 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, oxysterols, 22(R) hydroxycholesterol, 24(S) hydroxycholesterol, 25-epoxycholesterol, T0901317, 5-alpha-6-alpha-epoxycholesterol-3-sulfate (ECHS), 7-ketocholesterol-3-sulfate, famesol, bile acids, 1,1-biphosphonate esters, juvenile hormone III, and the like. Examples of diacylhydrazine ligands useful in the present invention include RG-115819 (3,5-Dimethyl-benzoic acid N-(1-ethyl-2,2-dimethyl-propyl)-N'-(2-methyl-3-methoxybenzoyl)-hydrazide), RG-115932 ((R)-3,5-Dimethyl-benzoic acid N-(1-tert-butyl-butyl)N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide), and RG-115830 (3,5-Dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide). *See, e.g.,* WO 2008/153801 (PCT/US2008/006757); and, WO 2013/036758 (PCT/US2012/054141), both of which are incorporated herein by reference in their entireties.

[0342] For example, a ligand for the ecdysone receptor-based gene switch may be selected from any suitable ligands. Both naturally occurring ecdysone or ecdysone analogs (e.g., 20-hydroxyecdysone, muristerone A, ponasterone A, ponasterone B, ponasterone C, 26-iodoponasterone A, inokosterone or 26-mesylinokosterone) and non-steroid inducers may be used as a ligand for gene switch of the present invention. U.S. Pat. No. 6,379,945, describes an insect steroid receptor isolated from *Heliothis virescens* ("HEcR") which is capable of acting as a gene switch responsive to both steroid and certain non-steroidal inducers. Non-steroidal inducers have a distinct advantage over steroids, in this and many other systems which are responsive to both steroids and non-steroid inducers, for several reasons including, for example: lower manufacturing cost, metabolic stability, absence from insects, plants, or mammals, and environmental acceptability. U.S. Pat. No. 6,379,945 describes the utility of two dibenzoylhydrazines, 1,2-dibenzoyl-1-tert-butyl-hydrazine and tebufenozide (N-(4-ethylbenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butyl-hydrazine) as ligands for an ecdysone-based gene switch. Also included in the present invention as a ligand are other dibenzoylhydrazines, such as those disclosed in U.S. Pat. No. 5,117,057. Use of tebufenozide as a chemical ligand for the ecdysone receptor from *Drosophila melanogaster* is also disclosed in U.S. Pat. No. 6,147,282. Additional, non-limiting examples of ecdysone ligands are 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, a 1,2-diacyl hydrazine, an N'-substituted-N,N'-disubstituted hydrazine, a dibenzoylalkyl cyanohydrazine, an N-substituted-N-alkyl-N,N'-diaroyl hydrazine, an N-substituted-N-acyl-N-alkyl, carbonyl hydrazine or an N-aroyl-N'-alkylN'-aroyl hydrazine. (See U.S. Pat. No. 6,723,531).

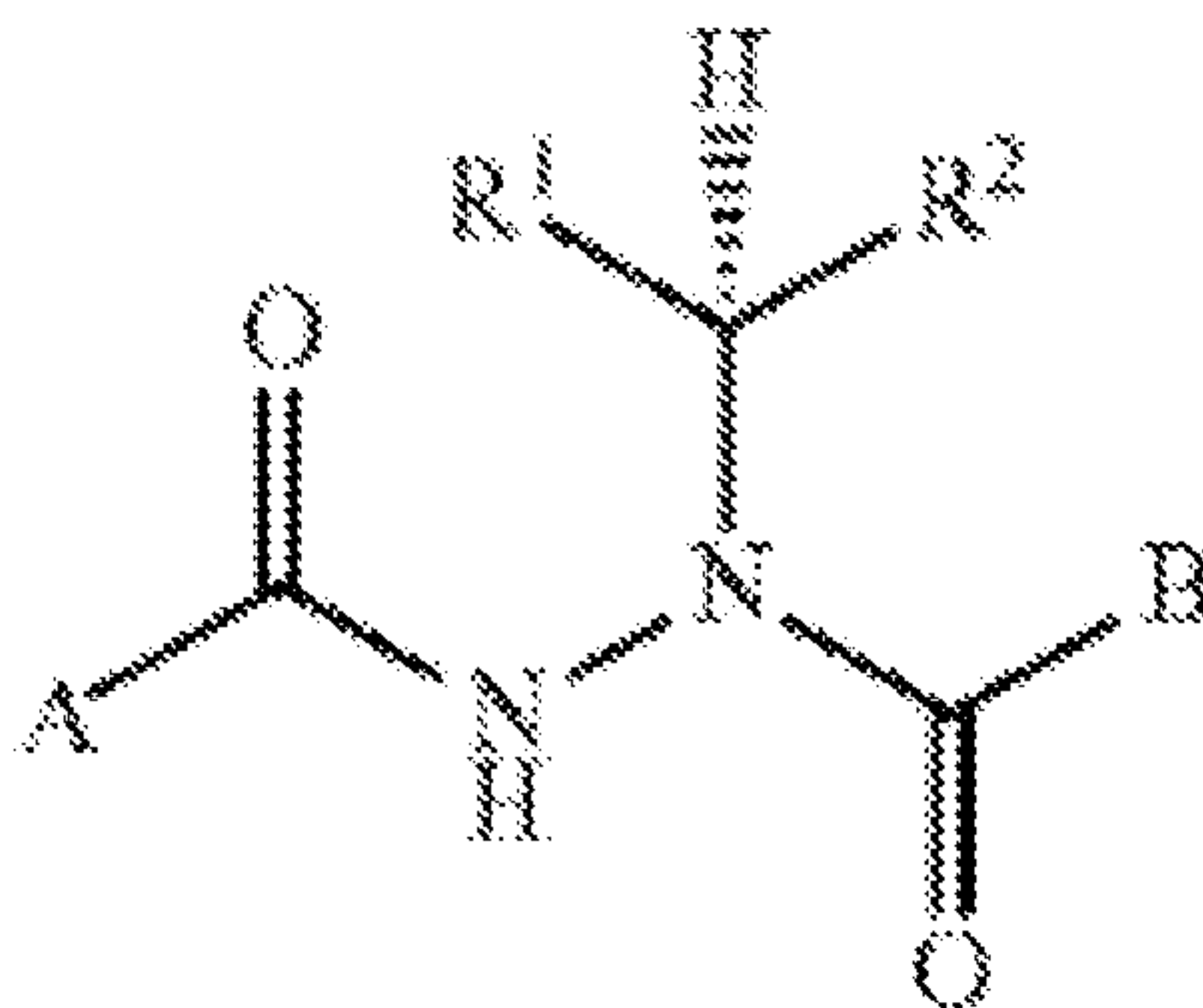
[0343] In one embodiment, the ligand for an ecdysone-based gene switch system is a diacylhydrazine ligand or chiral diacylhydrazine ligand. The ligand used in the gene switch system may be compounds of Formula I



Formula I

[0344] wherein A is alkoxy, arylalkyloxy or aryloxy; B is optionally substituted aryl or optionally substituted heteroaryl; and R1 and R2 are independently optionally substituted alkyl, arylalkyl, hydroxyalkyl, haloalkyl, optionally substituted cycloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heterocyclo, optionally substituted aryl or optionally substituted heteroaryl; or pharmaceutically acceptable salts, hydrates, crystalline forms or amorphous forms thereof.

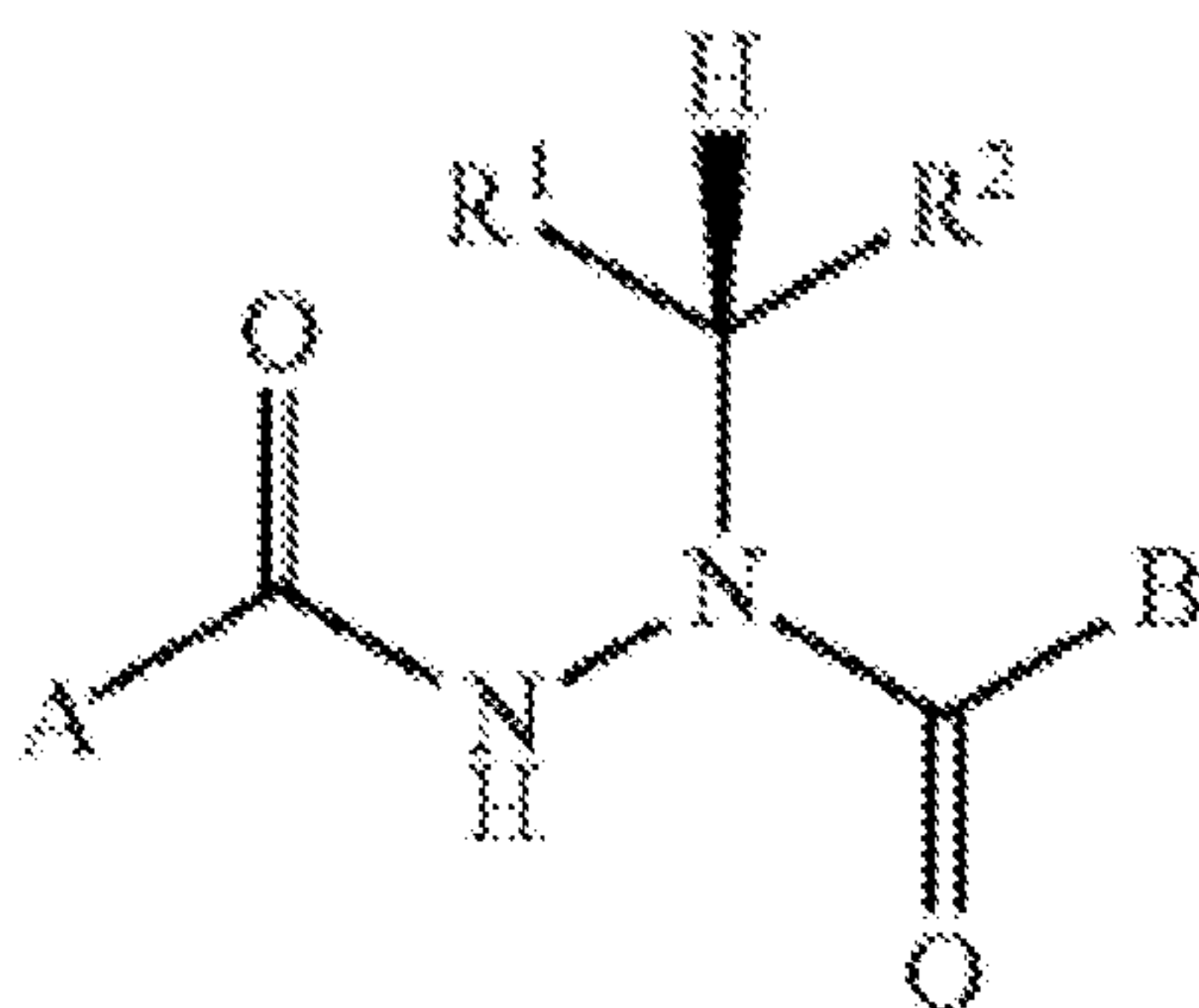
[0345] In another embodiment, the ligand may be enantiomerically enriched compounds of Formula II



Formula II

[0346] wherein A is alkoxy, arylalkyloxy, aryloxy, arylalkyl, optionally substituted aryl or optionally substituted heteroaryl; B is optionally substituted aryl or optionally substituted heteroaryl; and R1 and R2 are independently optionally substituted alkyl, arylalkyl, hydroxyalkyl, haloalkyl, optionally substituted cycloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heterocyclo, optionally substituted aryl or optionally substituted heteroaryl; with the proviso that R1 does not equal R2; wherein the absolute configuration at the asymmetric carbon atom bearing R1 and R2 is predominantly S; or pharmaceutically acceptable salts, hydrates, crystalline forms or amorphous forms thereof.

[0347] In certain embodiments, the ligand may be enantiomerically enriched compounds of Formula III



Formula III

[0348] wherein A is alkoxy, arylalkoxy, aryloxy, arylalkyl, optionally substituted aryl or optionally substituted heteroaryl; B is optionally substituted aryl or optionally substituted heteroaryl; and R1 and R2 are independently optionally substituted alkyl, arylalkyl, hydroxyalkyl, haloalkyl, optionally substituted cycloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heterocyclo, optionally substituted aryl or optionally substituted heteroaryl; with the proviso that R1 does not equal R2; wherein the absolute configuration at the asymmetric carbon atom bearing R1 and R2 is predominantly R; or pharmaceutically acceptable salts, hydrates, crystalline forms or amorphous forms thereof.

[0349] In one embodiment, a ligand may be (R)-3,5-dimethyl-benzoic acid N-(1-tertbutyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide having an enantiomeric excess of at least 95% or a pharmaceutically acceptable salt, hydrate, crystalline form or amorphous form thereof.

[0350] The diacylhydrazine ligands of Formula I and chiral diacylhydrazine ligands of Formula II or III, when used with an ecdysone-based gene switch system, provide the means for external temporal regulation of expression of a therapeutic polypeptide or therapeutic polynucleotide of the present invention. See U.S. Patent Nos.: 8,076,517; 8,884,060; and, 9,598,355; each of which are fully incorporated by reference herein.

[0351] The ligands used in the present invention may form salts. The term “salt(s)” as used herein denotes acidic and/or basic salts formed with inorganic and/or organic acids and bases. In addition, when a compound of Formula I, II or III contains both a basic moiety and an acidic moiety, zwitterions (“inner salts”) may be formed and are included within the term “salt(s)” as used herein. Pharmaceutically acceptable (i.e., non-toxic, physiologically acceptable) salts are used, although other salts are also useful, e.g., in isolation or

purification steps which may be employed during preparation. Salts of the compounds of Formula I, II or III may be formed, for example, by reacting a compound with an amount of acid or base, such as an equivalent amount, in a medium such as one in which the salt precipitates or in an aqueous medium followed by lyophilization.

[0352] The ligands which contain a basic moiety may form salts with a variety of organic and inorganic acids. Exemplary acid addition salts include acetates (such as those formed with acetic acid or trihaloacetic acid, for example, trifluoroacetic acid), adipates, alginates, ascorbates, aspartates, benzoates, benzenesulfonates, bisulfates, borates, butyrates, citrates, camphorates, camphorsulfonates, cyclopentanepropionates, digluconates, dodecylsulfates, ethanesulfonates, fumarates, glucoheptanoates, glycerophosphates, hemisulfates, heptanoates, hexanoates, hydrochlorides (formed with hydrochloric acid), hydrobromides (formed with hydrogen bromide), hydroiodides, 2-hydroxyethanesulfonates, lactates, maleates (formed with maleic acid), methanesulfonates (formed with methanesulfonic acid), 2-naphthalenesulfonates, nicotines, nitrates, oxalates, pectinates, persulfates, 3-phenylpropionates, phosphates, picrates, pivalates, propionates, salicylates, succinates, sulfates (such as those formed with sulfuric acid), sulfonates (such as those mentioned herein), tartrates, thiocyanates, toluenesulfonates such as tosylates, undecanoates, and the like.

[0353] The ligands which contain an acidic moiety may form salts with a variety of organic and inorganic bases. Exemplary basic salts include ammonium salts, alkali metal salts such as sodium, lithium, and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases (for example, organic amines) such as benzathines, dicyclohexylamines, hydrabamines (formed with N,N-bis(dehydroabietyl)ethylenediamine), N-methyl-D-glucamines, N-methyl-D-glucamides, t-butyl amines, and salts with amino acids such as arginine, lysine and the like.

[0354] Non-limiting examples of the ligands for the inducible gene expression system also includes those utilizing the FK506 binding domain are FK506, Cyclosporin A, or Rapamycin. FK506, rapamycin, and their analogs are disclosed in U.S. Pat. Nos.: 6,649,595; 6,187,757; 7,276,498; and, 7,273,874.

[0355] In some embodiments, a diacylhydrazine ligand for inducible gene expression is administered at unit daily dose of about 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or 120 mg. In some embodiments, the diacylhydrazine ligand is administered at a unit daily dose of about 5 mg. In some embodiments, the diacylhydrazine ligand is administered at a unit daily dose of about 10 mg. In some embodiments, the diacylhydrazine ligand is

administered at a unit daily dose of about 15 mg. In some embodiments, the diacylhydrazine ligand is administered daily at a unit daily dose of about 20 mg.

- [0356] In some embodiments, the combination therapy with two or more therapeutic agents can use agents that work by different mechanisms of action, although this is not required. Combination therapy using agents with different mechanisms of action can result in additive or synergetic effects. Combination therapy can allow for a lower dose of each agent than is used in monotherapy, thereby reducing toxic side effects and/or increasing the therapeutic index of the agent(s). Combination therapy can decrease the likelihood that resistant cancer cells will develop. In some embodiments, combination therapy comprises a therapeutic agent that affects the immune response (*e.g.*, enhances or activates the response) and a therapeutic agent that affects (*e.g.*, inhibits or kills) the tumor/cancer cells.
- [0357] In certain embodiments, in addition to administering the fusion protein or a fragment or a variant thereof described herein, the method or treatment further comprises administering at least one additional therapeutic agent. An additional therapeutic agent can be administered prior to, concurrently with, and/or subsequently to, administration of the agent. In some embodiments, the at least one additional therapeutic agent comprises 1, 2, 3, or more additional therapeutic agents.
- [0358] Therapeutic agents that can be administered in combination with the fusion protein or a fragment or a variant thereof described herein include chemotherapeutic agents. Thus, in some embodiments, the method or treatment involves the administration of an agent described herein in combination with a chemotherapeutic agent or in combination with a cocktail of chemotherapeutic agents. In some embodiments, the methods can further comprise one or more antineoplastic agents, such as cisplatin, capecitabine, or 5-fluorouracil, in combination with the fusion protein or a fragment or a variant thereof as described herein. Treatment with an agent can occur prior to, concurrently with, or subsequent to administration of chemotherapies. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously. Preparation and dosing schedules for such chemotherapeutic agents can be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *The Chemotherapy Source Book*, 4th Edition, 2008, M. C. Perry, Editor, Lippincott, Williams & Wilkins, Philadelphia, PA.

[0359] Useful classes of chemotherapeutic agents include, for example, anti-tubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (*e.g.*, platinum complexes such as cisplatin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, anti-folates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like. In certain embodiments, the second therapeutic agent is an alkylating agent, an antimetabolite, an antimetabolic, a topoisomerase inhibitor, or an angiogenesis inhibitor.

[0360] The fusion protein or a fragment or a variant thereof provided herein can be used alone or in combination with conventional therapeutic regimens such as surgery, irradiation, chemotherapy and/or bone marrow transplantation (autologous, syngeneic, allogeneic or unrelated). A set of tumor antigens can be useful, *e.g.*, in a large fraction of cancer patients.

Fusion Proteins in Combination with Chimeric Receptors

[0361] In some embodiments, the fusion protein is administered as a combination therapy with an additional therapeutic agent. In some embodiments, an additional therapeutic agent comprises a chimeric receptor, such as chimeric antigen receptor or an engineered T-cell receptor. For example, treatment can involve the simultaneous administration of the fusion protein with a chimeric receptor. In one embodiment, the treatment can involve the co-administration of a fusion protein and a modified effector cell comprising a chimeric receptor. In one embodiment, the treatment can involve the sequential administration of a modified effector cell which comprises a chimeric receptor followed by the administration of the fusion protein. In another embodiment, the treatment can involve the sequential administration of a fusion protein followed by the administration of a modified effector cell which comprises a chimeric receptor. In one aspect, there can be a lag of at least 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 20, or 24 hours between administrations. In another aspect, there can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 75, 90 or more days in between administrations.

[0362] In some embodiments, modified immune effector cells are modified immune cells that comprise T cells and/or natural killer cells. T cells or T lymphocytes are a subtype of white blood cells that are involved in cell-mediated immunity. Exemplary T cells include T

helper cells, cytotoxic T cells, TH17 cells, stem memory T cells (TSCM), naïve T cells, memory T cells, effector T cells, regulatory T cells, or natural killer T cells. In certain aspects, the embodiments described herein include making and/or expanding the modified immune effector cells (e.g., T-cells, Tregs, NK-cell or NK T-cells) that comprises transfecting the cells with an expression vector containing a DNA (or RNA) construct encoding the chimeric receptor.

[0363] In some embodiments, described herein includes a modified effector cell which comprises a chimeric receptor expressed on the surface of the cell. In some instances, the chimeric receptor comprises an antigen binding region that enables recognition and binding to a tumor antigen, e.g., a tumor-associated antigen or a tumor-specific antigen. In some instances, the antigen binding region comprises an antibody or binding fragment, for example, an Fab, an Fab', an F(ab')₂, an F(ab')₃, an scFv, an sc(Fv)₂, a dsFv, a diabody, a minibody, and a nanobody or binding fragments thereof. In some cases, the antigen binding region comprises a scFv. In some cases, the chimeric receptor comprises a scFv (e.g., a chimeric antigen receptor (CAR)). In some instances, the chimeric antigen receptor comprises a pattern-recognition receptor. In other cases, the chimeric receptor comprises an engineered T-cell receptor (TCR).

[0364] Further provided herein is an immune effector cell comprising a cell tag for use as a kill switch, selection marker, a biomarker, or a combination thereof. In some embodiments, the cell tag comprises HER1t, HER1t-1, CD20t-1 or CD20. In some cases, the cell tag comprises HER1t, and said HER1t comprises the polypeptide sequence of SEQ ID NO: 68. In some instances, the cell tag comprises HER1t-1, and said HER1t-1 comprises the polypeptide sequence of SEQ ID NO: 69.

Chimeric Antigen Receptors (CARs)

[0365] A chimeric antigen receptor (CAR) is an engineered receptor which grafts an exogenous specificity onto an immune effector cell. In some instances, a CAR comprises an extracellular domain (ectodomain) that comprises an antigen binding domain, a stalk region, a transmembrane domain and an intracellular (endodomain) domain. In some instances, the intracellular domain further comprises one or more intracellular signaling domains. In some instances, a CAR described herein comprises an antigen binding domain, a stalk region, a transmembrane domain, one or more costimulatory domains, and a signaling domain for T-cell activation.

[0366] An antigen binding domain can comprise complementary determining regions of a monoclonal antibody, variable regions of a monoclonal antibody, and/or antigen binding

fragments thereof. A complementarity determining region (CDR) is a short amino acid sequence found in the variable domains of antigen receptor (e.g., immunoglobulin and T-cell receptor) proteins that complements an antigen and therefore provides the receptor with its specificity for that particular antigen. Each polypeptide chain of an antigen receptor can contain three CDRs (CDR1, CDR2, and CDR3). In some instances, an antigen binding domain comprises F(ab')₂, Fab', Fab, Fv, or scFv. In some cases, an antigen binding domain is a scFv. In some cases, an antigen binding domain is a Fab. In some cases, an antigen binding domain is a Fab'. In some cases, an antigen binding domain is F(ab')₂. In some cases, an antigen binding domain is a Fv.

[0367] In some embodiments, a CAR described herein 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-a2, KDR, EDB-F, mesothelin, CD22, EGFR, Folate receptor α , Mucins such as MUC-1, MUC-4 or MUC-16, MAGE-A1, h5T4, PSMA, TAG-72, EGFR, CD20, EGFRvIII, CD123 or VEGF-R2. In one embodiment, a CAR described herein comprises an antigen binding domain that binds to an epitope on MUC16. In some embodiments, a CAR described herein comprises an antigen binding domain that binds to an epitope on CD19 or CD33. In some instances, a CAR described herein comprises an antigen binding domain that binds to an epitope on CD19. In some cases, a CAR described herein comprises an antigen binding domain that binds to an epitope on CD33. In further embodiments, a CAR described herein comprises an autoantigen or an antigen binding region that binds to an epitope on HLA-A2, myelin oligodendrocyte glycoprotein (MOG), factor VIII (FVIII), MAdCAM1, SDF1, or collagen type II.

[0368] In some embodiments, the CARs and methods described herein can be used for the treatment of a hyperproliferative disease, such as a cancer, an autoimmune disease or for the treatment of an infection, such as a viral, bacterial or parasitic infection. In some aspects, the CAR targets an antigen that is elevated in cancer cells, in autoimmune cells or in cells that are infected by a virus, bacteria or parasite. Pathogens that may be targeted include, without limitation, Plasmodium, trypanosome, Aspergillus, Candida, Hepatitis A, Hepatitis B, Hepatitis C, HSV, HPV, RSV, EBV, CMV, JC virus, BK virus, or Ebola pathogens. Autoimmune diseases can include graft-versus-host disease, rheumatoid arthritis, lupus, celiac disease, Crohn's disease, Sjogren Syndrome, polymyalgia rheumatic, multiple sclerosis, neuromyelitis optica, ankylosing spondylitis, Type 1 diabetes, alopecia areata,

vasculitis, temporal arteritis, bullous pemphigoid, psoriasis, pemphigus vulgaris, or autoimmune uveitis.

[0369] The pathogen recognized by a CAR may be essentially any kind of pathogen, but in some embodiments the pathogen is a fungus, bacteria, or virus. Exemplary viral pathogens include those of the families of Adenoviridae, Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Respiratory Syncytial Virus (RSV), JC virus, BK virus, HPV, HSV, HHV family of viruses, Hepatitis family of viruses, Picornaviridae, Herpesviridae, Hepadnaviridae, Flaviviridae, Retroviridae, Orthomyxoviridae, Paramyxoviridae, Papovaviridae, Polyomavirus, Rhabdoviridae, and Togaviridae. Exemplary pathogenic viruses cause smallpox, influenza, mumps, measles, chickenpox, ebola, and rubella. Exemplary pathogenic fungi include *Candida*, *Aspergillus*, *Cryptococcus*, *Histoplasma*, *Pneumocystis*, and *Stachybotrys*. Exemplary pathogenic bacteria include *Streptococcus*, *Pseudomonas*, *Shigella*, *Campylobacter*, *Staphylococcus*, *Helicobacter*, *E. coli*, *Rickettsia*, *Bacillus*, *Bordetella*, *Chlamydia*, *Spirochetes*, and *Salmonella*. In some embodiments the pathogen receptor Dectin-1 may be used to generate a CAR that recognizes the carbohydrate structure on the cell wall of fungi such as *Aspergillus*. In another embodiment, CARs can be made based on an antibody recognizing viral determinants (*e.g.*, the glycoproteins from CMV and Ebola) to interrupt viral infections and pathology.

[0370] In some embodiments, a “stalk” region, or a “spacer” or “hinge” region, is used to link the antigen-binding domain to the transmembrane domain. In some instances, a “stalk domain” or “stalk region” comprise any oligonucleotide- or polypeptide that functions to link the transmembrane domain to, either the extracellular domain or, the cytoplasmic domain in the polypeptide chain. In some embodiments, it is flexible enough to allow the antigen-binding domain to orient in different directions to facilitate antigen recognition. In some instances, the stalk region comprises the hinge region from IgG1. In alternative instances, the stalk region comprises the CH2CH3 region of immunoglobulin and optionally portions of CD3. In some cases, the stalk region comprises a CD8 α hinge region, an IgG4-Fc 12 amino acid hinge region (ESKYGPPCPPCP) or IgG4 hinge regions as described in WO/2016/073755.

[0371] The transmembrane domain can be derived from either a natural or a synthetic source. Where the source is natural, the domain can be derived from any membrane-bound or transmembrane protein. Suitable transmembrane domains can include the transmembrane region(s) of alpha, beta or zeta chain of the T-cell receptor; or a transmembrane region from CD28, CD3 epsilon, CD3 ζ , CD45, CD4, CD5, CD8alpha, CD9, CD16, CD22, CD33, CD37,

CD64, CD80, CD86, CD134, CD137 or CD154. Alternatively the transmembrane domain can be synthetic, and can comprise hydrophobic residues such as leucine and valine. In some embodiments, a triplet of phenylalanine, tryptophan and valine is found at one or both termini of a synthetic transmembrane domain. Optionally, a short oligonucleotide or polypeptide linker, in some embodiments, between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic signaling domain of a CAR. In some embodiments, the linker is a glycine-serine linker.

[0372] In some embodiments, the transmembrane domain comprises a CD8 α transmembrane domain or a CD3 ζ transmembrane domain. In some embodiments, the transmembrane domain comprises a CD8 α transmembrane domain. In other embodiments, the transmembrane domain comprises a CD3 ζ transmembrane domain.

[0373] The intracellular domain can comprise one or more costimulatory domains. Exemplary costimulatory domains include, but are not limited to, CD8, CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134) or fragment or combination thereof. In some instances, a CAR described herein comprises one or more, or two or more of costimulatory domains selected from CD8, CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134) or fragment or combination thereof. In some instances, a CAR described herein comprises one or more, or two or more of costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, OX40 (CD134) or fragment or combination thereof. In some instances, a CAR described herein comprises one or more, or two or more of costimulatory domains selected from CD8, CD28, 4-1BB (CD137), or fragment or combination thereof. In some instances, a CAR described herein comprises one or more, or two or more of costimulatory domains selected from CD28, 4-1BB (CD137), or fragment or combination thereof. In some instances, a CAR described herein comprises costimulatory domains CD28 and 4-1BB (CD137) or their respective fragments thereof. In some instances, a CAR described herein comprises costimulatory domains CD28 and OX40 (CD134) or their respective fragments thereof. In some instances, a CAR described herein comprises costimulatory domains CD8 and CD28 or their respective fragments thereof. In some instances, a CAR described herein comprises costimulatory domains CD28 or a fragment thereof. In some instances, a CAR described herein comprises costimulatory domains 4-1BB (CD137) or a fragment thereof. In some instances, a CAR described herein comprises costimulatory domains OX40 (CD134) or a fragment thereof. In some instances, a CAR described herein comprises costimulatory domains CD8 or a fragment thereof.

- [0374] In some embodiments, the intracellular domain further comprises a signaling domain for T-cell activation. In some instances, the signaling domain for T-cell activation comprises a domain derived from TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b or CD66d. In some cases, the signaling domain for T-cell activation comprises a domain derived from CD3 ζ .
- [0375] In some embodiments, a CAR described herein is administered to a subject with one or more additional therapeutic agents that include but are not limited to cytokines as described herein. In further embodiments, an immune effector cell expressing a CAR described herein expresses membrane-bound IL-15 ("mIL-15 or mbIL-15"). In aspects of the invention, the mbIL-15 comprises a fusion protein between IL-15 and IL-15R α . In further embodiments, the mbIL-15 comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 69. In certain cases, the CAR and the cytokine is expressed in separate vectors. In specific cases, the vectors can be lentiviral vectors, retroviral vectors or Sleeping Beauty transposons.

CD19-Specific CARs

- [0376] CD19 is a cell surface glycoprotein of the immunoglobulin superfamily and is found predominately in malignant B-lineage cells. In some instances, CD19 has also been detected in solid tumors such as pancreatic cancer, liver cancer, and prostate cancer.
- [0377] In some embodiments, described herein include a CD19-specific CAR, in which the antigen binding domain comprises a F(ab')₂, Fab', Fab, Fv, or scFv. In some instances, the antigen binding domain recognizes an epitope on CD19. In some embodiments, the antigen binding domain recognizes an epitope on CD19 that is also recognized by FMC63. In some embodiments the scFv and/or VH/VL domains is derived from FMC63. FMC63 generally refers to a mouse monoclonal IgG1 antibody raised against Nalm-1 and -16 cells expressing CD19 of human origin (Ling, N. R., et al. (1987). Leucocyte typing III. 302). In some instances, the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3 ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3 ζ .
- [0378] In some embodiments, the antigen binding domain recognizes an epitope on CD19 that is also recognized by JCAR014, JCAR015, JCAR017, or 19-28z CAR (Juno Therapeutics). In some embodiments, described herein include a CD19-specific CAR-T cell, in which the antigen binding domain recognizes an epitope on CD19 that is also recognized by JCAR014, JCAR015, JCAR017, or 19-28z CAR (Juno Therapeutics). In some instances,

the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3ζ.

[0379] In some embodiments, described herein include a CD19-specific CAR-T cell comprises a scFv antigen binding domain, and the antigen binding domain recognizes an epitope on CD19 that is also recognized by JCAR014, JCAR015, JCAR017, or 19-28z CAR (Juno Therapeutics). In some instances, the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3ζ.

[0380] In some embodiments, a CD19-specific CAR-T cell described herein comprises an anti-CD19 antibody described in US20160152723. In some embodiments, a CD19-specific CAR-T cell described herein comprises an anti-CD19 antibody described in WO2015/123642. In some embodiments, a CD19-specific CAR-T cell described herein comprises an anti-CD19 scFv derived from clone FMC63 (Nicholson et al. Construction and characterisation of a functional CD19 specific single chain Fv fragment for immunotherapy of B lineage leukaemia and lymphoma. Mol. Immunol., 34:1157-1165, 1997).

[0381] In some embodiments, the antigen binding domain recognizes an epitope on CD19 that is also recognized by KTE-C19 (Kite Pharma, Inc.). In some embodiments, described herein include a CD19-specific CAR-T cell, in which the antigen binding domain recognizes an epitope on CD19 that is also recognized by KTE-C19. In some instances, the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3ζ.

[0382] In some embodiments, described herein include a CD19-specific CAR-T cell comprises a scFv antigen binding domain, and the antigen binding domain recognizes an epitope on CD19 that is also recognized by KTE-C19. In some instances, the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3ζ.

- [0383] In some embodiments, a CD19-specific CAR-T cell described herein comprises an anti-CD19 antibody described in WO2015187528 or fragment or derivative thereof.
- [0384] In some embodiments, the antigen binding domain recognizes an epitope on CD19 that is also recognized by CTL019 (Novartis). In some embodiments, described herein include a CD19-specific CAR-T cell, in which the antigen binding domain recognizes an epitope on CD19 that is also recognized by CTL019. In some instances, the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3ζ.
- [0385] In some embodiments, described herein include a CD19-specific CAR-T cell comprises a scFv antigen binding domain, and the antigen binding domain recognizes an epitope on CD19 that is also recognized by CTL019. In some instances, the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3ζ.
- [0386] In some embodiments, the antigen binding domain recognizes an epitope on CD19 that is also recognized by UCART19 (Cellestis). In some embodiments, described herein include a CD19-specific CAR-T cell, in which the antigen binding domain recognizes an epitope on CD19 that is also recognized by UCART19. In some instances, the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3ζ.
- [0387] In some embodiments, described herein include a CD19-specific CAR-T cell comprises a scFv antigen binding domain, and the antigen binding domain recognizes an epitope on CD19 that is also recognized by UCART19. In some instances, the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3ζ.
- [0388] In some embodiments, the antigen binding domain recognizes an epitope on CD19 that is also recognized by BPX-401 (Bellicum). In some embodiments, described herein

include a CD19-specific CAR-T cell, in which the antigen binding domain recognizes an epitope on CD19 that is also recognized by BPX-401. In some instances, the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3ζ.

[0389] In some embodiments, described herein include a CD19-specific CAR-T cell comprises a scFv antigen binding domain, and the antigen binding domain recognizes an epitope on CD19 that is also recognized by BPX-401. In some instances, the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3ζ.

[0390] In some cases, the antigen binding domain recognizes an epitope on CD19 that is also recognized by blinatumomab (Amgen), coltuximabravtansine (ImmunoGen Inc./Sanofi-aventis), MOR208 (Morphosys AG/Xencor Inc.), MEDI-551 (Medimmune), denintuzumabmafodotin (Seattle Genetics), B4 (or DI-B4) (Merck Serono), taplitumomabpaptox (National Cancer Institute), XmAb 5871 (Amgen/Xencor, Inc.), MDX-1342 (Medarex) or AFM11 (Affimed). In some instances, the CD19-specific CAR further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3ζ.

[0391] In some embodiments, described herein include a CD19-specific CAR-T cell, in which the antigen binding domain comprises a F(ab')₂, Fab', Fab, Fv, or scFv. In some instances, the antigen binding domain recognizes an epitope on CD19. In some cases, the antigen binding domain recognizes an epitope on CD19 that is also recognized by blinatumomab (Amgen), coltuximabravtansine (ImmunoGen Inc./Sanofi-aventis), MOR208 (Morphosys AG/Xencor Inc.), MEDI-551 (Medimmune), denintuzumabmafodotin (Seattle Genetics), B4 (or DI-B4) (Merck Serono), taplitumomabpaptox (National Cancer Institute), XmAb 5871 (Amgen/Xencor, Inc.), MDX-1342 (Medarex) or AFM11 (Affimed). In some instances, the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10,

DAP12, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3 ζ .

[0392] In some cases, a CD19-specific CAR-T cell described herein comprise a scFv antigen binding domain, and the antigen binding domain recognizes an epitope on CD19 that is also recognized by FMC63, blinatumomab (Amgen), coltuximabravtansine (ImmunoGen Inc./Sanofi-aventis), MOR208 (Morphosys AG/Xencor Inc.), MEDI-551 (Medimmune), denintuzumabmafodotin (Seattle Genetics), B4 (or DI-B4) (Merck Serono), taplitumomabpaptox (National Cancer Institute), XmAb 5871 (Amgen/Xencor, Inc.), MDX-1342 (Medarex) or AFM11 (Affimed). In some instances, the CD19-specific CAR-T cell further comprises a transmembrane domain selected from a CD8alpha transmembrane domain or a CD3 ζ transmembrane domain; one or more costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134) or fragment or combination thereof; and a signaling domain from CD3 ζ .

CD33-Specific CARs

[0393] "CD33," is a 67kDa single pass transmembrane glycoprotein and is a member of the sialic acid-binding immunoglobulin-like lectins (Siglecs) super-family. CD33 is characterized by a V-set Ig-like domain responsible for sialic acid binding and a C2-set Ig-like domain in its extracellular domain. Alternative splicing of CD33 mRNA leads to a shorter isoform (CD33m) lacking the V-set Ig-like domain as well as the disulfide bond linking the V- and C2-set Ig-like domains. In healthy subjects, CD33 is primarily expressed as a myeloid differentiation antigen found on normal multipotent myeloid precursors, unipotent colony-forming cells, monocytes and maturing granulocytes. CD33 is expressed on more than 80% of myeloid leukemia cells but not on normal hematopoietic stem cells or mature granulocytes. (Andrews, R. et al., The L4F3 antigen is expressed by unipotent and multipotent colony-forming cells but not by their precursors, *Blood*, 68(5):1030-5 (1986)). CD33 has been reported to be expressed on malignant myeloid cells, activated T cells and activated NK cells and is found on at least a subset of blasts in the vast majority of AML patients (Pollard, J. et al., Correlation of CD33 expression level with disease characteristics and response to gemtuzumab ozogamicin containing chemotherapy in childhood AML, *Blood*, 119(16):3705-11 (2012)). In addition to broad expression on AML blasts, CD33 may be expressed on stem cells underlying AML.

[0394] In embodiments, the antigen binding moiety of a CAR described herein is specific to CD33 (CD33 CAR). The CD33-specific CAR, when expressed on the cell surface, redirects the specificity of T cells to human CD33. In embodiments, the antigen binding domain

comprises a single chain antibody fragment (scFv) comprising a variable domain light chain (VL) and variable domain heavy chain (VH) of a target antigen specific monoclonal anti-CD33 antibody joined by a flexible linker, such as a glycine-serine linker or a Whitlow linker. In embodiments, the scFv are M195, m2H12, DRB2, and/or My9-6. In embodiments, the scFv is humanized, for example, hM195. In some embodiments, the antigen binding moiety may comprise VH and VL that are directionally linked, for example, from N to C terminus, VH-linker-VL or VL-linker-VH.

[0395] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:35 (hM195 VL).

[0396] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:36 (hM195 VH).

[0397] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:37 (M2H12 VH).

[0398] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:38 (M2H12 VL).

[0399] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:39 (DRB2 VH).

[0400] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:40 (DRB2 VL).

[0401] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:41 (My9-6 VH).

[0402] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:42 (My9-6 VL).

MUC16-Specific CARs

[0403] MUC16 is a large carbohydrate antigen, also known as CA-125. MUC16 is encoded by the MUC16 gene located on human chromosome 19. MUC16 is a highly glycosylated

multi-domain type I transmembrane protein comprising 3 domains. The C-terminal domain contains multiple extracellular SEA (sea urchin sperm protein, enterokinase, and agrin) modules that have an autoproteolytic activity. SEA harbors two proteolytic sites proximal to the transmembrane (TM) domain. A large cleaved domain termed CA-125 is released into circulation at acidic pH. CA-125 is commonly used as disease biomarker for ovarian cancer. The highly conserved truncated extracellular membrane tethered protein domain called MUC16ecto domain. A MUC16 antibody was identified that specifically bound the ectodomain of MUC16 that is retained on the tumor cell surface. “Overexpression of MUC16” by a cell of interest (such as a cancer cell) refers to a higher level of MUC16 protein and/or mRNA that is expressed by the cell of interest compared to a control cell (such as a non-cancerous cell, normal cell, etc.).

[0404] In embodiments, the antigen binding moiety of a CAR described herein is specific to MUC16 (MUC16 CAR). The MUC16-specific CAR, when expressed on the cell surface, redirects the specificity of T cells to human MUC16. In embodiments, the antigen binding domain comprises a single chain antibody fragment (scFv) comprising a variable domain light chain (VL) and variable domain heavy chain (VH) of a target antigen specific monoclonal anti- MUC16 antibody joined by a flexible linker, such as a glycine-serine linker or a Whitlow linker. In embodiments, the scFv are MUC16-1 scFv (SEQ ID NOs: 43-44), MUC16-2 scFv (SEQ ID NOs: 45-46), MUC16-3 scFv (SEQ ID NOs: 47-48), MUC16-4 scFv (SEQ ID NOs: 49-50), MUC16-5 scFv (SEQ ID NOs: 51-52), MUC16-6 scFv (SEQ ID NOs: 53-54) or MUC16-7 scFv (SEQ ID NOs: 55-56). In embodiments, the scFv is humanized.

[0405] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:43 (MUC16-1).

[0406] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:44 (MUC16-1).

[0407] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:45 (MUC16-2).

[0408] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:46 (MUC16-2).

- [0409] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:47 (MUC16-3).
- [0410] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:48 (MUC16-3).
- [0411] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:49 (MUC16-4).
- [0412] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:50 (MUC16-4).
- [0413] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:51 (MUC16-5).
- [0414] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:52 (MUC16-5).
- [0415] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:53 (MUC16-5).
- [0416] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:54 (MUC16-6).
- [0417] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:55 (MUC16-7).
- [0418] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:56 (MUC16-7).
- [0419] In some embodiments, the antigen binding moiety can comprise VH and VL that are directionally linked, for example, from N to C terminus, VH-linker-VL or VL-linker-VH.

Engineered T-cell Receptor (TCR)

[0420] In some embodiments, the chimeric receptor comprises an engineered T-cell receptor.

The T cell receptor (TCR) is composed of two chains ($\alpha\beta$ or $\gamma\delta$) that pair on the surface of the T cell to form a heterodimeric receptor. In some instances, the $\alpha\beta$ TCR is expressed on most T cells in the body and is known to be involved in the recognition of specific MHC-restricted antigens. Each α and β chain are composed of two domains: a constant domain (C) which anchors the protein to the cell membrane and is associated with invariant subunits of the CD3 signaling apparatus; and a variable domain (V) that confers antigen recognition through six loops, referred to as complementarity determining regions (CDRs). In some instances, each of the V domains comprises three CDRs; e.g., CDR1, CDR2 and CDR3 with CDR3 as the hypervariable region. These CDRs interact with a complex formed between an antigenic peptide bound to a protein encoded by the major histocompatibility complex (pepMHC) (e.g., HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, or HLA-DRB1 complex). In some instances, the constant domain further comprises a joining region that connects the constant domain to the variable domain. In some cases, the beta chain further comprises a short diversity region which makes up part of the joining region.

[0421] In some cases, such TCR are reactive to specific tumor antigen, e.g. NY-ESO, Mage A3, Titin. In other cases, such TCR are reactive to specific neoantigens expressed within a patient's tumor (i.e. patient-specific, somatic, non-synonymous mutations expressed by tumors). In some cases, engineered TCRs can be affinity-enhanced.

[0422] In some embodiments, a TCR is described using the International Immunogenetics (IMGT) TCR nomenclature, and links to the IMGT public database of TCR sequences. For example, there can be several types of alpha chain variable ($V\alpha$) regions and several types of beta chain variable ($V\beta$) regions distinguished by their framework, CDR1, CDR2, and CDR3 sequences. As such, a $V\alpha$ type can be referred to in IMGT nomenclature by a unique TRAV number. For example, "TRAV21" defines a TCR $V\alpha$ region having unique framework and CDR1 and CDR2 sequences, and a CDR3 sequence which is partly defined by an amino acid sequence which is preserved from TCR to TCR but which also includes an amino acid sequence which varies from TCR to TCR. Similarly, "TRBV5-1" defines a TCR $V\beta$ region having unique framework and CDR1 and CDR2 sequences, but with only a partly defined CDR3 sequence.

[0423] In some cases, the beta chain diversity region is referred to in IMGT nomenclature by the abbreviation TRBD.

- [0424] In some instances, the unique sequences defined by the IMGT nomenclature are widely known and accessible to those working in the TCR field. For example, they can be found in the IMGT public database and in “T cell Receptor Factsbook”, (2001) LeFranc and LeFranc, Academic Press, ISBN 0-12-441352-8.
- [0425] In some embodiments, an $\alpha\beta$ heterodimeric TCR is, for example, transfected as full length chains having both cytoplasmic and transmembrane domains. In some cases, the TCRs contain an introduced disulfide bond between residues of the respective constant domains, as described, for example, in WO 2006/000830.
- [0426] In some instances, TCRs described herein are in single chain format, for example see WO 2004/033685. Single chain formats include $\alpha\beta$ TCR polypeptides of the $V\alpha$ -L- $V\beta$, $V\beta$ -L- $V\alpha$, $V\alpha$ -C α -L- $V\beta$, $V\alpha$ -L- $V\beta$ -C β , $V\alpha$ -C α -L- $V\beta$ -C β types, wherein $V\alpha$ and $V\beta$ are TCR α and β variable regions respectively, C α and C β are TCR α and β constant regions respectively, and L is a linker sequence. In certain embodiments single chain TCRs of the invention may have an introduced disulfide bond between residues of the respective constant domains, as described in WO 2004/033685.
- [0427] The TCR described herein may be associated with a detectable label, a therapeutic agent or a PK modifying moiety.
- [0428] Exemplary detectable labels for diagnostic purposes include, but are not limited to, fluorescent labels, radiolabels, enzymes, nucleic acid probes and contrast reagents.
- [0429] Therapeutic agents which may be associated with the TCRs described herein include immunomodulators, radioactive compounds, enzymes (perforin for example) or chemotherapeutic agents. To ensure that toxic effects are exercised in the desired location the toxin could be inside a liposome linked to a TCR so that the compound is released in a controlled manner. In some cases, the controlled release minimize damaging effects during the transport in the body and ensure that the toxin has maximum effect after binding of the TCR to the relevant antigen presenting cells.
- [0430] In some embodiments, additional suitable therapeutic agents include for instance:
- a. small molecule cytotoxic agents, e.g., compounds with the ability to kill mammalian cells having a molecular weight of less than 700 Daltons. Such compounds could also contain toxic metals capable of having a cytotoxic effect. Furthermore, it is to be understood that these small molecule cytotoxic agents also include pro-drugs, i.e. compounds that decay or are converted under physiological conditions to release cytotoxic agents. Examples of such agents include cisplatin, maytansine derivatives, rachelmycin, calicheamicin, docetaxel, etoposide,

gemcitabine, ifosfamide, irinotecan, melphalan, mitoxantrone, sorfimer sodiumphotofrin II, temozolomide, topotecan, trimetreate glucuronate, auristatin E vincristine and doxorubicin;

- b. peptide cytotoxins, i.e. proteins or fragments thereof with the ability to kill mammalian cells. For example, ricin, diphtheria toxin, pseudomonas bacterial exotoxin A, DNase and RNase;
- c. radio-nuclides, i.e. unstable isotopes of elements which decay with the concurrent emission of one or more of α or β particles, or γ rays. For example, iodine 131, rhenium 186, indium 111, yttrium 90, bismuth 210 and 213, actinium 225 and astatine 213; chelating agents may be used to facilitate the association of these radio-nuclides to the high affinity TCRs, or multimers thereof;
- d. immuno-stimulants, i.e. immune effector molecules which stimulate immune response. For example, cytokines such as IL-2 and IFN- γ ,
- e. superantigens and mutants thereof;
- f. TCR-HLA fusions;
- g. chemokines such as IL-8, platelet factor 4, melanoma growth stimulatory protein, etc;
- h. antibodies or fragments thereof, including anti-T cell or NK cell determinant antibodies (e.g. anti-CD3, anti-CD28 or anti-CD16);
- i. alternative protein scaffolds with antibody like binding characteristics
- j. complement activators; and
- k. xenogeneic protein domains, allogeneic protein domains, viral/bacterial protein domains, viral/bacterial peptides.

Doses

[0431] Suitable dosages of the fusion protein and modified immune effector cells used will depend on the age and weight of the subject and the particular drug used. Dosages and therapeutic regimens of the fusion protein and modified immune effector cells can be determined by a skilled artisan.

[0432] In certain embodiments, the fusion protein is administered by injection (e.g., subcutaneously or intravenously) at a dose of about 1 to 30 mg/kg, e.g., about 5 to 25 mg/kg, about 10 to 20 mg/kg, about 1 to 5 mg/kg, or about 3 mg/kg. In some embodiments, the fusion protein is administered at a dose of about 1 mg/kg, about 3 mg/kg, about 5 mg/kg, about 10 mg/kg, about 20 mg/kg, about 30 mg/kg, or about 40 mg/kg. In some embodiments, the fusion protein is administered at a dose of about 1-3 mg/kg, or about 3-10 mg/kg. In some embodiments, the fusion protein is administered at a dose of about 0.5-2, 2-4, 2-5, 5-15, or 5-20 mg/kg. The dosing schedule can vary from e.g., once a week to once every 2, 3,

or 4 weeks. In one embodiment, the fusion protein is administered at a dose from about 10 to 20 mg/kg every other week. In another embodiment, the fusion protein is administered at a dose of about 1 mg/kg once every two weeks, about 3 mg/kg once every two weeks, 10 mg/kg once every two weeks, 3 mg/kg once every four weeks, or 5 mg/kg once every four weeks.

[0433] In other embodiments, the fusion protein is administered by injection (e.g., subcutaneously or intravenously) at a dose (e.g., a flat dose) of about 200 mg to 500 mg, e.g., about 250 mg to 450 mg, about 300 mg to 400 mg, about 250 mg to 350 mg, about 350 mg to 450 mg, or about 300 mg or about 400 mg. In some embodiments, the fusion protein is administered at a dose of about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, or about 500 mg. In some embodiments, the fusion protein is administered at a dose of 200 or 300 mg. In some embodiments, the fusion protein is administered at a dose of about 250-450 mg, or about 300-400 mg. In some embodiments, the fusion protein is administered at a dose of about 200-300 mg, 250-350 mg, 300-400 mg, 350-450 mg, or 400-500 mg. The dosing schedule can vary from e.g., once a week to once every 2, 3, 4, 5, or 6 weeks. In one embodiment the fusion protein is administered at a dose from about 300 mg to 400 mg once every three or once every four weeks. In one embodiment, the fusion protein is administered at a dose from about 300 mg once every three weeks. In one embodiment, the fusion protein is administered at a dose from about 400 mg once every four weeks. In one embodiment, the fusion protein is administered at a dose from about 300 mg once every four weeks. In one embodiment, the fusion protein is administered at a dose from about 400 mg once every three weeks. The fusion protein can be administered one or more times, e.g., one, two, three, four, five, six, seven or more times. In one embodiment, the fusion protein is administered six times. The fusion protein can be administered at least 5 days, e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 20, 25, 30, 35, or 40 days, after administration of CAR-expressing cells, e.g., MUC16, CD33, CD19 or BCMA - specific CAR expressing cells. In some embodiments, the fusion protein can be administered about 8 days or about 15 days after administration of CAR-expressing cells, e.g., MUC16 specific CAR expressing cells or CD33 specific CAR expressing cells.

[0434] The fusion protein can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is intravenous injection or infusion. For example, the fusion protein can be administered by intravenous infusion at a rate of more than 20 mg/min, e.g., 20-40 mg/min, and typically greater than or equal to 40 mg/min to reach a dose of about 35 to 440 mg/m², typically about

70 to 310 mg/m², and more typically, about 110 to 130 mg/m². In embodiments, the fusion protein can be administered by intravenous infusion at a rate of less than 10 mg/min; preferably less than or equal to 5 mg/min to reach a dose of about 1 to 100 mg/m², preferably about 5 to 50 mg/m², about 7 to 25 mg/m² and more preferably, about 10 mg/m².

[0435] The fusion protein can be administered by intravenous infusion at a rate of more than 20 mg/min, e.g., 20-40 mg/min, and typically greater than or equal to 40 mg/min to reach a dose of about 35 to 440 mg/m², typically about 70 to 310 mg/m², and more typically, about 110 to 130 mg/m². In embodiments, the infusion rate of about 110 to 130 mg/m² achieves a level of about 3 mg/kg. In other embodiments, the fusion protein can be administered by intravenous infusion at a rate of less than 10 mg/min, e.g., less than or equal to 5 mg/min to reach a dose of about 1 to 100 mg/m², e.g., about 5 to 50 mg/m², about 7 to 25 mg/m², or, about 10 mg/m². In some embodiments, the antibody is infused over a period of about 30 min.

Modified Effector Cell Doses

[0436] In some embodiments, an amount of modified effector cells is administered to a subject in need thereof and the amount is determined based on the efficacy and the potential of inducing a cytokine-associated toxicity. In some cases, an amount of modified effector cells comprises about 10⁵ to about 10⁹ modified effector cells/kg. In some cases, an amount of modified effector cells comprises about 10⁵ to about 10⁸ modified effector cells/kg. In some cases, an amount of modified effector cells comprises about 10⁵ to about 10⁷ modified effector cells/kg. In some cases, an amount of modified effector cells comprises about 10⁶ to about 10⁹ modified effector cells/kg. In some cases, an amount of modified effector cells comprises about 10⁶ to about 10⁸ modified effector cells/kg. In some cases, an amount of modified effector cells comprises about 10⁷ to about 10⁹ modified effector cells/kg. In some cases, an amount of modified effector cells comprises about 10⁵ to about 10⁶ modified effector cells/kg. In some cases, an amount of modified effector cells comprises about 10⁶ to about 10⁷ modified effector cells/kg. In some cases, an amount of modified effector cells comprises about 10⁷ to about 10⁸ modified effector cells/kg. In some cases, an amount of modified effector cells comprises about 10⁸ to about 10⁹ modified effector cells/kg. In some instances, an amount of modified effector cells comprises about 10⁹ modified effector cells/kg. In some instances, an amount of modified effector cells comprises about 10⁸ modified effector cells/kg. In some instances, an amount of modified effector cells comprises about 10⁷ modified effector cells/kg. In some instances, an amount of modified

effector cells comprises about 10^6 modified effector cells/kg. In some instances, an amount of modified effector cells comprises about 10^5 modified effector cells/kg.

[0437] In some embodiments, the modified effector cells are modified T cells. In some instances, the modified T cells are CAR-T cells. In some cases, an amount of CAR-T cells comprises about 10^5 to about 10^9 CAR-T cells/kg. In some cases, an amount of CAR-T cells comprises about 10^5 to about 10^8 CAR-T cells/kg. In some cases, an amount of CAR-T cells comprises about 10^5 to about 10^7 CAR-T cells/kg. In some cases, an amount of CAR-T cells comprises about 10^6 to about 10^9 CAR-T cells/kg. In some cases, an amount of CAR-T cells comprises about 10^6 to about 10^8 CAR-T cells/kg. In some cases, an amount of CAR-T cells comprises about 10^7 to about 10^9 CAR-T cells/kg. In some cases, an amount of CAR-T cells comprises about 10^5 to about 10^6 CAR-T cells/kg. In some cases, an amount of CAR-T cells comprises about 10^6 to about 10^7 CAR-T cells/kg. In some cases, an amount of CAR-T cells comprises about 10^7 to about 10^8 CAR-T cells/kg. In some cases, an amount of CAR-T cells comprises about 10^8 to about 10^9 CAR-T cells/kg. In some instances, an amount of CAR-T cells comprises about 10^9 CAR-T cells/kg. In some instances, an amount of CAR-T cells comprises about 10^8 CAR-T cells/kg. In some instances, an amount of CAR-T cells comprises about 10^7 CAR-T cells/kg. In some instances, an amount of CAR-T cells comprises about 10^6 CAR-T cells/kg. In some instances, an amount of CAR-T cells comprises about 10^5 CAR-T cells/kg.

[0438] In some embodiments, the CAR-T cells are CD19-specific CAR-T cells. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^5 to about 10^9 CAR-T cells/kg. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^5 to about 10^8 CAR-T cells/kg. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^5 to about 10^7 CAR-T cells/kg. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^6 to about 10^9 CAR-T cells/kg. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^6 to about 10^8 CAR-T cells/kg. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^7 to about 10^9 CAR-T cells/kg. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^5 to about 10^6 CAR-T cells/kg. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^6 to about 10^7 CAR-T cells/kg. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^7 to about 10^8 CAR-T cells/kg. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^8 to about 10^9 CAR-T cells/kg. In some instances, an amount of CD19-specific CAR-T cells comprises about 10^9 CAR-T cells/kg. In some instances, an amount of CD19-specific CAR-T cells comprises about 10^8

CAR-T cells/kg. In some instances, an amount of CD19-specific CAR-T cells comprises about 10^7 CAR-T cells/kg. In some instances, an amount of CD19-specific CAR-T cells comprises about 10^6 CAR-T cells/kg. In some instances, an amount of CD19-specific CAR-T cells comprises about 10^5 CAR-T cells/kg.

[0439] In some embodiments, the modified T cells are engineered TCR T-cells. In some cases, an amount of engineered TCR T- cells comprises about 10^5 to about 10^9 TCR cells/kg. In some cases, an amount of engineered TCR cells comprises about 10^5 to about 10^8 TCR cells/kg. In some cases, an amount of engineered TCR cells comprises about 10^5 to about 10^7 TCR cells/kg. In some cases, an amount of engineered TCR cells comprises about 10^6 to about 10^9 TCR cells/kg. In some cases, an amount of engineered TCR cells comprises about 10^6 to about 10^8 TCR cells/kg. In some cases, an amount of engineered TCR cells comprises about 10^7 to about 10^9 TCR cells/kg. In some cases, an amount of engineered TCR cells comprises about 10^5 to about 10^6 TCR cells/kg. In some cases, an amount of engineered TCR cells comprises about 10^6 to about 10^7 TCR cells/kg. In some cases, an amount of engineered TCR cells comprises about 10^7 to about 10^8 TCR cells/kg. In some cases, an amount of engineered TCR cells comprises about 10^8 to about 10^9 TCR cells/kg. In some instances, an amount of engineered TCR cells comprises about 10^9 TCR cells/kg. In some instances, an amount of engineered TCR cells comprises about 10^8 TCR cells/kg. In some instances, an amount of engineered TCR cells comprises about 10^7 TCR cells/kg. In some instances, an amount of engineered TCR cells comprises about 10^6 TCR cells/kg. In some instances, an amount of engineered TCR cells comprises about 10^5 TCR cells/kg.

[0440] It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

Expression and Purification System of Fusion Proteins

[0441] The fusion protein or a fragment or a variant thereof described herein can be produced from cells by culturing a host cell transformed with the expression vector comprising one or more polynucleotides encoding the fusion protein or a fragment or a variant thereof, under conditions, and for an amount of time, sufficient to allow expression of the fusion protein, or a fragment or a variant thereof. For example, polypeptides expressed in *E. coli* can be

refolded from inclusion bodies (*see, e.g.*, Hou et al. (1998) *Cytokine* 10:319-30). Bacterial expression systems and methods for their use are well known in the art (*see* Current Protocols in Molecular Biology, Wiley & Sons, and Molecular Cloning – A Laboratory Manual – 3rd Ed., Cold Spring Harbor Laboratory Press, New York (2001)). The choice of codons, suitable expression vectors and suitable host cells can vary depending on a number of factors, and can be easily optimized as needed. The fusion protein or a fragment or a variant thereof described herein can be expressed in mammalian cells or in other expression systems including but not limited to yeast, baculovirus, and *in vitro* expression systems (*see, e.g.*, Kaszubska et al. (2000) *Protein Expression and Purification* 18:213-220).

- [0442] Following expression, the fusion protein or a fragment or a variant thereof can be purified or isolated. The term “purified” or “isolated” as applied to any of the fusion proteins or fragments or variants thereof described herein can refer to a polypeptide or a protein that has been separated or purified from components (*e.g.*, proteins or other naturally-occurring biological or organic molecules) which naturally accompany it, *e.g.*, other proteins, lipids, and nucleic acid in a prokaryote expressing the proteins. Typically, a polypeptide is purified when it constitutes at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9% by weight of the total protein in a sample.
- [0443] The fusion protein or a fragment or a variant thereof described herein can be isolated or purified in a variety of ways depending on what other components are present in the sample. Standard purification methods include electrophoretic, molecular, immunological, and chromatographic techniques, including ion exchange, hydrophobic, affinity, and reverse-phase HPLC chromatography. For example, the fusion protein or a fragment or a variant thereof can be purified using a standard anti-fusion protein antibody affinity column. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. *See, e.g.*, Scopes (1994) “Protein Purification, 3rd edition,” Springer-Verlag, New York City, N.Y. The degree of purification necessary can vary depending on the desired use. In some instances, no purification of the expressed fusion protein or a fragment or variant thereof is necessary.
- [0444] Methods for determining the yield or purity of a purified fusion protein or a fragment or a variant thereof can include, *e.g.*, Bradford assay, UV spectroscopy, Biuret protein assay, Lowry protein assay, amido black protein assay, high pressure liquid chromatography (HPLC), mass spectrometry (MS), and gel electrophoretic methods (*e.g.*, using a protein

stain such as Coomassie Blue or colloidal silver stain). Once expressed, purified, or after being purified following expression, a fusion protein or a fragment or a variant thereof described herein can be assayed for any one of a number of desired properties using *in vitro* or *in vivo* assays such as any of those described herein. For example, a fusion protein or a fragment or a variant thereof described herein can be assayed for e.g., its ability to inhibit PD-1 and trap TGF- β .

- [0445] The fusion protein or a fragment or a variant thereof described herein can be produced using a variety of techniques. For example, a polynucleotide encoding a fusion protein or a fragment or a variant thereof described herein can be inserted into an expression vector that contains transcriptional and translational regulatory sequences, which can include, *e.g.*, promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, transcription terminator signals, polyadenylation signals, and enhancer or activator sequences. In some embodiments, the regulatory sequences can include a promoter and transcriptional start and stop sequences. In addition, the expression vector can include more than one replication system such that it can be maintained in two different organisms, for example, in mammalian or insect cells for expression and in a prokaryotic host for cloning and amplification.
- [0446] Several possible vector systems are available for the expression of the fusion protein or a fragment or a variant thereof from nucleic acids in mammalian cells (*e.g.*, host cells). For example, one class of vectors relies upon the integration of the desired gene sequences into the host cell genome. Cells which have stably integrated DNA can be selected by simultaneously introducing drug resistance genes such as *E. coli* gpt (Mulligan and Berg (1981) *Proc Natl Acad Sci USA* 78:2072) or Tn5 neo (Southern and Berg (1982) *Mol Appl Genet* 1:327). The selectable marker gene can be either linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection (Wigler et al. (1979) *Cell* 16:77). Another class of vectors utilizes DNA elements which confer autonomously replicating capabilities to an extrachromosomal plasmid. These vectors can be derived from animal viruses, such as bovine papillomavirus (Sarver et al. (1982) *Proc Natl Acad Sci USA*, 79:7147), polyoma virus (Deans et al. (1984) *Proc Natl Acad Sci USA* 81:1292), or SV40 virus (Lusky and Botchan (1981) *Nature* 293:79). The expression vectors can be introduced into cells (*e.g.*, host cells) in a manner suitable for subsequent expression of the nucleic acid. The method of introduction is largely dictated by the targeted cell type, discussed below. Non-limiting exemplary methods can include CaPO₄ precipitation, liposome fusion,

lipofectin, electroporation, viral infection, dextran-mediated transfection, polybrene-mediated transfection, protoplast fusion, and direct microinjection.

[0447] Appropriate host cells for the expression of the fusion protein or a fragment or a variant thereof can include, but not limited to, yeast, bacteria, insect, plant, and, as described above, mammalian cells. Of interest are bacteria such as *E. coli*, fungi such as *Saccharomyces cerevisiae* and *Pichia pastoris*, insect cells such as SF9, mammalian cell lines (e.g., human cell lines), as well as primary cell lines (e.g., primary mammalian cells). In some embodiments, the fusion protein or a fragment or a variant thereof can be expressed in Chinese hamster ovary (CHO) cells or in a suitable myeloma cell line such as (NS0). Suitable cell lines also include, for example, BHK-21 (baby hamster kidney) cells; 293 (human embryonic kidney) cells; HMEpC (Human Mammary Epithelial cells; 3T3 (mouse embryonic fibroblast) cells.

[0448] The methods described herein provide for the expression and purification of the fusion protein or a fragment or a variant thereof in various cell-based expression systems, such as protein production in bacterial, mammalian, insect, yeast, and chymadomonas cells. Protein expression can be constitutive or inducible with inducers such as copper sulfate, sugars such as galactose, methanol, methylamine, thiamine, tetracycline, or IPTG. After the fusion protein or a fragment or a variant thereof is expressed in the host cells, the host cells are lysed to liberate the fusion protein or a fragment or a variant thereof for purification. Methods of lysing the various host cells are featured in “Sample Preparation-Tools for Protein Research” EMD Bioscience and in the Current Protocols in Protein Sciences (CPPS). A non-limiting exemplary purification method is affinity chromatography, such as ion-metal affinity chromatograph using nickel, cobalt, or zinc affinity resins for e.g., a histidine-tagged fusion protein or fragment or variant thereof. Methods of purifying histidine-tagged proteins are described by Clontech using their Talonx cobalt resin and by Novagen in their pET system manual, 10th edition. Another non-limiting exemplary purification strategy is by immuno-affinity chromatography, for example, anti-myc antibody conjugated resin can be used to affinity purify e.g., a myc-tagged fusion protein or fragment or variant thereof. Enzymatic digestion with serine proteases, such as thrombin and enterokinase, cleave and release the fusion protein or a fragment or a variant thereof from the histidine or myc tag, releasing the fusion protein or a fragment or a variant thereof from the affinity resin while the histidine-tags and myc-tags are left attached to the affinity resin.

[0449] Methods of introducing and expressing polynucleotides encoding the fusion protein or a fragment or a variant thereof into a cell (e.g., a host cell) are known in the art. In the

context of an expression vector, the vector comprising the polynucleotide encoding the fusion protein or a fragment or a variant thereof can be readily introduced into a host cell, *e.g.*, mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

- [0450] Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (2001)). In embodiments, a method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection or polyethylenimine (PEI) Transfection.
- [0451] Biological methods for introducing polynucleotides encoding the fusion protein or a fragment or a variant thereof into a host cell can include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, *e.g.*, human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.
- [0452] In some embodiments, the expression vector can have the necessary 5' upstream and 3' downstream regulatory elements such as promoter sequences, ribosome recognition and binding TATA box, and 3' UTR AAUAAA transcription termination sequence for the efficient gene transcription and translation in its respective host cell. The expression vector can have additional sequence such as 6X-histidine, V5, thioredoxin, glutathione-S-transferase, c-Myc, VSV-G, HSV, FLAG, maltose binding peptide, metal-binding peptide, HA and "secretion" signals (Honeybee melittin, α -factor, PHO, Bip), which are incorporated into the expressed fusion protein or fragment or variant thereof. In addition, there can be enzyme digestion sites incorporated after these sequences to facilitate enzymatic removal of them after they are not needed. These additional sequences are useful for the detection of the fusion protein expression, for protein purification by affinity chromatography, enhanced solubility of the recombinant protein in the host cytoplasm, and/or for secreting the fusion protein out into the culture media, into the periplasm of the prokaryote bacteria, or the spheroplast of the yeast cells. The expression of the fusion protein can be constitutive in the host cells or it can be induced, *e.g.*, with copper sulfate, sugars, such as galactose, methanol, methylamine, thiamine, tetracycline, infection with

baculovirus, and (isopropyl-beta-D-thiogalactopyranoside) IPTG, a stable synthetic analog of lactose.

[0453] Non-limiting examples of expression vectors and host cells can include the pET vectors (Novagen), pGEX vectors (Amersham Pharmacia), and pMAL vectors (New England labs. Inc.) for protein expression in *E. coli* host cell such as BL21, BL21(DE3) and AD494(DE3)pLysS, Rosetta (DE3), and Origami(DE3) (Novagen); the strong CMV promoter-based pcDNA3.1 (Invitrogen) and pCIneo vectors (Promega) for expression in mammalian cell lines such as CHO, COS, HEK-293, Jurkat, and MCF-7; replication incompetent adenoviral vector vectors pAdeno X, pAd5F35, pLP-Adeno-X-CMV (Clontech), pAd/CMV/V5-DEST, pAd-DEST vector (Invitrogen) for adenovirus-mediated gene transfer and expression in mammalian cells; pLNCX2, pLXSN, and pLAPSN retrovirus vectors for use with the Retro-XTM system from Clontech for retroviral-mediated gene transfer and expression in mammalian cells; pLenti4V5-DEST.TM., pLenti6/V5-DESTTM, and pLenti6.2/V5-GW/lacZ (Invitrogen) for lentivirus-mediated gene transfer and expression in mammalian cells; adenovirus-associated virus expression vectors such as pAAV-MCS, pAAV-IRES-hrGFP, and pAAV-RC vector (Stratagene) for adeno-associated virus-mediated gene transfer and expression in mammalian cells; BACpak6 baculovirus (Clontech) and pFastBacTM-HT (Invitrogen) for the expression in *Spodopora frugiperda* 9 (S9) and Sf11 insect cell lines; pMT/BiP/V5-His (Invitrogen) for the expression in *Drosophila* Schneider S2 cells; Pichia expression vectors pPICZ α , pPICZ, pFLD α and pFLD (Invitrogen) for expression in *Pichia pastoris* and vectors pMET α and pMET for expression in *P. methanolica*; pYES2/GS and pYD1 (Invitrogen) vectors for expression in yeast *Saccharomyces cerevisiae*. Recent advances in the large scale expression heterologous proteins in *Chlamydomonas reinhardtii* are described by Griesbeck C. et. al. 2006 Mol. Biotechnol. 34:213-33 and Fuhrmann M. 2004, Methods Mol Med. 94:191-5.

[0454] Besides cell-based expression systems, cell-free expression systems are also contemplated. Cell-free expression systems offer several advantages over traditional cell-based expression methods, including the easy modification of reaction conditions to favor protein folding, decreased sensitivity to product toxicity and suitability for high-throughput strategies such as rapid expression screening or large amount protein production because of reduced reaction volumes and process time. The cell-free expression system can use plasmid or linear DNA. Moreover, improvements in translation efficiency have resulted in yields that exceed a milligram of protein per milliliter of reaction mix.

- [0455] In one embodiment, a continuous cell-free translation system can be used to produce the fusion protein or a fragment or a variant thereof. A continuous cell-free translation system capable of producing proteins in high yield is described by Spirin A S. et. al., Science 242:1162 (1988). The method uses a continuous flow design of the feeding buffer which contains amino acids, adenosine triphosphate (ATP), and guanosine triphosphate (GTP) throughout the reaction mixture and a continuous removal of the translated polypeptide product. The system uses *E. coli* lysate to provide the cell-free continuous feeding buffer. This continuous flow system is compatible with both prokaryotic and eukaryotic expression vectors. Large scale cell-free production is described by Chang G. et. al., Science 310:1950-3 (2005).
- [0456] Other commercially available cell-free expression systems include the Expressway™ Cell-Free Expression Systems (Invitrogen), which utilize an *E. coli*-based *in vitro* system for efficient, coupled transcription and translation reactions to produce up to milligram quantities of active recombinant protein in a tube reaction format; the Rapid Translation System (RTS) (Roche Applied Science), which also uses an *E. coli*-based *in vitro* system; and the TNT Coupled Reticulocyte Lysate Systems (Promega), which uses rabbit reticulocyte-based in-vitro system.
- [0457] In other exemplary production methods, the fusion protein or a fragment or a variant thereof described herein can be synthesized *de novo* in whole or in part, using chemical methods. For example, the component amino acid sequences of the fusion protein or a fragment or a variant thereof can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography followed by chemical linkage to form a desired polypeptide. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing.
- [0458] Methods for detecting and/or measuring the amount of endotoxin present in a sample (both before and after purification) can be based on commercial kits that are available. For example, the concentration of endotoxin in a protein sample can be determined using the QCL-1000 Chromogenic kit (BioWhittaker), the *limulus amoebocyte* lysate (LAL)-based kits such as the Pyrotell®, Pyrotell®-T, Pyrochrome®, Chromo-LAL, and CSE kits available from the Associates of Cape Cod Incorporated. In some embodiments, endotoxins can be removed from the fusion protein preparations using a variety of commercially available reagents including, without limitation, the ProteoSpin™ Endotoxin Removal Kits (Norgen Biotek Corporation), Detoxi-Gel Endotoxin Removal Gel (Thermo Scientific; Pierce Protein

Research Products), MiraCLEAN® Endotoxin Removal Kit (Mirus), or Acrodisc™-Mustang® E membrane (Pall Corporation).

[0459] In some embodiments, following expression and purification, the fusion protein or a fragment or a variant thereof described herein can be modified. The modifications can be covalent or non-covalent modifications. Such modifications can be introduced into the fusion protein or a fragment or a variant thereof by, *e.g.*, reacting targeted amino acid residues of the fusion protein or a fragment or a variant thereof with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. Suitable sites for modification can be chosen using any of a variety of criteria including, *e.g.*, structural analysis or amino acid sequence analysis of the fusion protein or a fragment or a variant thereof.

[0460] In some exemplary production methods, the fusion protein or a fragment or a variant thereof as described herein can be conjugated to a heterologous moiety. In some embodiments, the heterologous moiety can be, *e.g.*, a heterologous polypeptide, a therapeutic agent (*e.g.*, a toxin or a drug), or a detectable label such as, but not limited to, a radioactive label, an enzymatic label, a fluorescent label, or a luminescent label. Suitable heterologous polypeptides can include, *e.g.*, an antigenic tag (*e.g.*, FLAG, polyhistidine, hemagglutinin (HA), glutathione-S-transferase (GST), or maltose-binding protein (MBP)) for use in purifying antibodies or fragments or variants thereof. Heterologous polypeptides can also include polypeptides that are useful as diagnostic or detectable markers, for example, luciferase, green fluorescent protein (GFP), or chloramphenicol acetyl transferase (CAT). Where the heterologous moiety is a polypeptide, the moiety can be incorporated into the fusion protein or a fragment or a variant thereof described herein, resulting in a fusion protein comprising the heterologous moiety.

Promoters

[0461] “Promoter” refers to a region of a polynucleotide that initiates transcription of a coding sequence. Promoters are located near the transcription start sites of genes, on the same strand and upstream on the DNA (towards the 5' region of the sense strand). Some promoters are constitutive as they are active in all circumstances in the cell, while others are regulated becoming active in response to specific stimuli, *e.g.*, an inducible promoter. Yet other promoters are tissue specific or activated promoters, including but not limited to T-cell specific promoters.

- [0462] The term “promoter activity” and its grammatical equivalents as used herein refer to the extent of expression of nucleotide sequence that is operably linked to the promoter whose activity is being measured. Promoter activity can be measured directly by determining the amount of RNA transcript produced, for example by Northern blot analysis or indirectly by determining the amount of product coded for by the linked nucleic acid sequence, such as a reporter nucleic acid sequence linked to the promoter.
- [0463] “Inducible promoter” as used herein refers to a promoter which is induced into activity by the presence or absence of transcriptional regulators, *e.g.*, biotic or abiotic factors. Inducible promoters are useful because the expression of genes operably linked to them can be turned on or off at certain stages of development of an organism or in a particular tissue. Examples of inducible promoters are alcohol-regulated promoters, tetracycline-regulated promoters, steroid-regulated promoters, metal-regulated promoters, pathogenesis-regulated promoters, temperature-regulated promoters and light-regulated promoters.
- [0464] One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. However, other constitutive promoter sequences can also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the present disclosure should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the present disclosure. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionein promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.
- [0465] In some embodiments, the promoter is a constitutive promoter, a tissue specific promoter, or an inducible promoter. In some embodiments, the inducible promoter is a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch.
- [0466] Additional promoter elements, *e.g.*, enhancers, can regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of

the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it can appear that individual elements can function either cooperatively or independently to activate transcription.

Reporter

[0467] Reporter genes can be used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the host cell and that encodes a polypeptide whose expression is manifested by some easily detectable property, *e.g.*, enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the polypeptides encoding the fusion protein or a fragment or a variant thereof has been introduced into the host cells. Suitable reporter genes can include, but not limited to, genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (*e.g.*, Ui-Tei et al., FEBS Letters 479: 79-82 (2000)). Suitable expression systems are well known and can be prepared using known techniques or obtained commercially. In general, a polynucleotide construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions can be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

Variants

[0468] The term "fragment" or "variant" refers to variants and derivatives of the fusion protein described herein, containing one or more binding moieties to *e.g.*, PD-1 and/or TGF- β . In certain embodiments, amino acid sequence variants of the fusion protein are contemplated. For example, in some embodiments, amino acid sequence variants of the fusion protein described herein are contemplated to improve the binding affinity and/or other biological properties of the fusion protein. Exemplary method for preparing amino acid variants include, but are not limited to, introducing appropriate modifications into the nucleotide sequence encoding the fusion protein, or by peptide synthesis. Such

modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the fusion protein.

[0469] Any combination of deletion, insertion, and substitution can be made to the various domains to arrive at the final fusion protein, provided that the final fusion protein possesses the desired characteristics, *e.g.*, PD-1 inhibition and TGF- β trap. In some embodiments, fusion protein variants having one or more amino acid substitutions are provided. In some embodiments, sites of interest for substitution mutagenesis can include the CDRs and framework regions. In some embodiments, amino acid substitutions can be introduced into the variable domains of the target-binding protein of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved antibody-dependent cell mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). Both conservative and non-conservative amino acid substitutions can be contemplated for preparing the antibody variants (*e.g.*, PD-1 antibody variants).

[0470] In another example of a substitution to create a variant fusion protein can be substituting one or more hypervariable region residues in a parent antibody. In general, variants are then selected based on improvements in desired properties compared to a parent antibody, for example, increased affinity, reduced affinity, reduced immunogenicity, increased pH dependence of binding. For example, an affinity matured variant antibody can be generated, *e.g.*, using phage display-based affinity maturation techniques, such as those described herein and known in the field. Substitutions can be made in hypervariable regions (HVR) of a parent antibody to generate variants, and variants are then selected based on binding affinity, *i.e.*, by affinity maturation. In some embodiments of affinity maturation, diversity can be introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library can be then created. The library can be then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity can involve HVR- directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) can be randomized. HVR residues involved in antigen binding can be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. Substitutions can be in one, two, three, four, or more sites within a parent antibody sequence.

[0471] In some embodiments, a fragment or a variant of the fusion protein, as described herein can comprise a VL and a VH domain with amino acid sequences corresponding to the amino acid sequence of a naturally occurring VL or VH domain, respectively, but that has been “humanized”, *i.e.*, by replacing one or more amino acid residues in the amino acid

sequence of the naturally occurring VL or VH domains (and in particular in the framework sequences) by one or more of the amino acid residues that occur at the corresponding position(s) in a VL or VH domain from a conventional 4-chain antibody from a human. This can be performed in a manner known in the field, which can be clear to the skilled person, for example on the basis of the further description herein. It should be noted that such humanized fusion protein or a fragment or a variant thereof are obtained in any suitable manner known per se and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring VL and/or VH domain as a starting material.

[0472] In some embodiments, a fragment or a variant of the fusion protein, as described herein, comprises a VL and a VH domain with amino acid sequences corresponding to the amino acid sequence of a naturally occurring VL or VH domain, respectively, but that has been “camelized”, *i.e.*, by replacing one or more amino acid residues in the amino acid sequence of a naturally occurring VL or VH domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a VL or a VH domain of a heavy chain antibody. Such “camelizing” can be preferably inserted at amino acid positions that form and/or are present at the VH-VL interface, and/or at the so-called *Camelidae* hallmark residues (see for example WO 94/04678 and Davies and Riechmann (1994 and 1996)). In some embodiments, the VH sequence that is used as a starting material or starting point for generating or designing the camelized single domain can be a VH sequence from a mammal, *e.g.*, a human, such as a VH3 sequence. It should be noted that such camelized fusion protein or a fragment or a variant thereof, in certain embodiments, can be obtained in any suitable manner known in the field and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring VL and/or VH domain as a starting material.

[0473] For example, both “humanization” and “camelization” can be performed by providing a nucleotide sequence that encodes a naturally occurring VL and/or VH domain, respectively, and then changing, one or more codons in the nucleotide sequence in such a way that the new polynucleotide sequence encodes a “humanized” or “camelized” fusion protein or a fragment or a variant thereof, respectively. This polynucleotide can then be expressed, so as to provide the desired binding capabilities (*e.g.*, PD-1). Alternatively, in other embodiments, the fusion protein or a fragment or a variant thereof comprises a “humanized” or “camelized” antibody synthesized *de novo* using known peptide synthesis technique from the amino acid sequence of a naturally occurring antibody comprising a VL

and/or VH domain. In some embodiments, the fusion protein or a fragment or a variant thereof comprises a “humanized” or “camelized” antibody synthesized *de novo* using known peptide synthesis technique from the amino acid sequence or nucleotide sequence of a naturally occurring antibody comprising a VL and/or VH domain, respectively, a nucleotide sequence encoding the desired humanized or camelized antibody of the disclosure, respectively, is designed and then synthesized *de novo* using known techniques for nucleic acid synthesis, after which the nucleic acid thus obtained is expressed in using known expression techniques, so as to provide the desired antibody.

[0474] Other suitable methods and techniques for obtaining the fusion protein or a fragment or a variant thereof, starting from naturally occurring sequences for VL or VH domains, for example, comprises combining one or more parts of one or more naturally occurring VL or VH sequences (such as one or more framework (FR) sequences and/or complementarity determining region (CDR) sequences), and/or one or more synthetic or semi-synthetic sequences, and/or a naturally occurring sequence for a CH2 domain, and a naturally occurring sequence for a CH3 domain comprising amino acid substitutions that favor formation of heterodimer over homodimer, in a suitable manner, so as to provide the fusion protein or a fragment or a variant thereof.

Antibody Engineering

[0475] In some embodiments, it can be desirable to change certain amino acids containing exposed side-chains to another amino acid residue in order to provide for greater chemical stability of a final antibody (*e.g.*, PD-1 antibody), as follows. The deamidation of asparagine can occur on N-G or D-G sequences and result in the creation of an isoaspartic acid residue that introduces a kink into the polypeptide chain and decreases its stability (isoaspartic acid effect). In some embodiments, the antibodies of the fusion protein of the present disclosure do not contain asparagine isomerism sites.

[0476] For example, an asparagine (Asn) residue may be changed to Gln or Ala to reduce the potential for formation of isoaspartate at any Asn-Gly sequences, particularly within a CDR. A similar problem can occur at a Asp-Gly sequence. Reissner and Aswad (2003) *Cell. Mol. Life Sci.* 60:1281. Isoaspartate formation can debilitate or completely abrogate binding of an antibody to its target antigen. See, Presta (2005) *J. Allergy Clin. Immunol.* 116:731 at 734. In one embodiment, the asparagine can be changed to glutamine (Gln). It can also be desirable to alter an amino acid adjacent to an asparagine (Asn) or glutamine (Gln) residue to reduce the likelihood of deamidation, which occurs at greater rates when small amino acids

occur adjacent to asparagine or glutamine. See, Bischoff & Kolbe (1994) J. Chromatog. 662:261. In addition, any methionine residues (typically solvent exposed Met) in CDRs can be changed to Lys, Leu, Ala, or Phe in order to reduce the possibility that the methionine sulfur would oxidize, which can reduce antigen binding affinity and also can contribute to molecular heterogeneity in the final antibody preparation. *Id.* In one embodiment, the methionine can be changed to alanine (Ala). Additionally, in order to prevent or minimize potential scissile Asn-Pro peptide bonds, it can be desirable to alter any Asn-Pro combinations found in a CDR to Gln-Pro, Ala-Pro, or Asn-Ala. Antibodies with such substitutions can be subsequently screened to ensure that the substitutions do not decrease the affinity or specificity of the antibody for human PD-L1, or other desired biological activity to unacceptable levels.

[0477] An antibody of the fusion protein disclosed herein can also be conjugated to a chemical moiety such as a radionuclide or other detectable label. Radionuclides include ^{99}Tc , ^{90}Y , ^{111}In , ^{32}P , ^{14}C , ^{125}I , ^3H , ^{131}I , ^{11}C , ^{15}O , ^{13}N , ^{18}F , ^{35}S , ^{51}Cr , ^{57}Co , ^{226}Ra , ^{60}Co , ^{59}Fe , ^{57}Se , ^{152}Eu , ^{67}Cu , ^{217}Bi , ^{211}At , ^{212}Pb , ^{47}Sc , ^{109}Pd , ^{234}Th , and ^{40}K , ^{157}Gd , ^{55}Mn , ^{52}Tr , and ^{56}Fe . Fluorescent or chemiluminescent labels can include fluorophores, such as rare earth chelates, fluorescein and its derivatives, rhodamine and its derivatives, isothiocyanate, phycoerythrin, phycocyanin, allophycocyanin, o-phthalaldehyde, fluorescamine, ^{152}Eu , dansyl, umbelliferone, luciferin, luminal label, isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, an aequorin label, 2,3-dihydrophthalazinediones, biotin/avidin, spin labels and stable free radicals.

[0478] Any method known in the art for conjugating antibody molecules to the various moieties can be employed, including those methods described by Hunter, et al., (1962) Nature 144:945; David, et al., (1974) Biochemistry 13:1014; Pain, et al., (1981) J. Immunol. Meth. 40:219; and Nygren, J., (1982) Histochem. and Cytochem. 30:407. Methods for conjugating antibodies are conventional and very well known in the art.

Pharmaceutical Compositions

[0479] The present disclosure provides a composition comprising the fusion protein or a fragment or a variant thereof described herein and a carrier therefor (*e.g.*, a pharmaceutically acceptable carrier). The composition desirably is a physiologically acceptable (*e.g.*, pharmaceutically acceptable) composition, which comprises a carrier, preferably a physiologically (*e.g.*, pharmaceutically) acceptable carrier, and the fusion protein or a fragment or a variant thereof. Any suitable carrier can be used within the context of the

present disclosure, and such carriers are well known in the art. The choice of carrier will be determined, in part, by the particular use of the composition (*e.g.*, administration to an animal) and the particular method used to administer the composition. Ideally, in the context of replication-deficient adenoviral vectors, the pharmaceutical composition preferably is free of replication-competent adenovirus. The pharmaceutical composition optionally can be sterile.

[0480] Suitable compositions include aqueous and non-aqueous isotonic sterile solutions, which can contain anti-oxidants, buffers, and bacteriostats, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The composition can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, immediately prior to use. Extemporaneous solutions and suspensions can be prepared from sterile powders, granules, and tablets. Preferably, the carrier is a buffered saline solution. In some cases, the fusion protein or a fragment or a variant thereof is part of a composition formulated to protect the fusion protein or a fragment or a variant thereof from damage prior to administration. For example, the composition can be formulated to reduce loss of the fusion protein or a fragment or a variant thereof on devices used to prepare, store, or administer the fusion protein or a fragment or a variant thereof, such as glassware, syringes, or needles. The composition can be formulated to decrease the light sensitivity and/or temperature sensitivity of the fusion protein or a fragment or a variant thereof. To this end, the composition preferably comprises a pharmaceutically acceptable liquid carrier, such as, for example, those described above, and a stabilizing agent selected from the group consisting of polysorbate 80, L-arginine, polyvinylpyrrolidone, trehalose, and combinations thereof. Use of such a composition will extend the shelf life of the fusion protein or a fragment or a variant thereof, and facilitate its administration. Formulations for fusion protein or a fragment or a variant thereof -containing compositions are further described in, for example, U.S. Patent 6,225,289, U.S. Patent 6,514,943, and International Patent Application Publication WO 2000/034444.

[0481] The composition also can be formulated to enhance transduction efficiency. In addition, one of ordinary skill in the art will appreciate that the fusion protein or a fragment or a variant thereof can be present in a composition with other therapeutic or biologically-active agents. For example, factors that control inflammation, such as ibuprofen or steroids, can be part of the composition to reduce swelling and inflammation associated with *in vivo*

administration of the fusion protein or a fragment or a variant thereof. Antibiotics, *i.e.*, microbicides and fungicides, can be present to treat existing infection and/or reduce the risk of future infection, such as infection associated with the administration procedures.

- [0482] In some instances, pharmaceutical compositions comprising the presently described fusion protein or a fragment or a variant thereof are formulated in a conventional manner using one or more physiologically acceptable carriers including excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. A summary of pharmaceutical compositions described herein is found, for example, in Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980; and Pharmaceutical Dosage Forms and Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins 1999).
- [0483] Pharmaceutical compositions are optionally manufactured in a conventional manner, such as, by way of example only, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or compression processes.
- [0484] In certain embodiments, compositions can also include one or more pH adjusting agents or buffering agents, including acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-hydroxymethylaminomethane; and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an acceptable range.
- [0485] In other embodiments, compositions can also include one or more salts in an amount required to bring osmolality of the composition into an acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions; suitable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite and ammonium sulfate.
- [0486] The pharmaceutical compositions described herein are administered by any suitable administration route, including but not limited to, oral, parenteral (*e.g.*, intravenous, subcutaneous, intramuscular, intracerebral, intracerebroventricular, intra-articular,

intraperitoneal, or intracranial), intranasal, buccal, sublingual, or rectal administration routes. In some instances, the pharmaceutical composition is formulated for parenteral (*e.g.*, intravenous, subcutaneous, intramuscular, intracerebral, intracerebroventricular, intra-articular, intraperitoneal, or intracranial) administration.

[0487] The pharmaceutical compositions described herein are formulated into any suitable dosage form, including but not limited to, aqueous oral dispersions, liquids, gels, syrups, elixirs, slurries, suspensions and the like, for oral ingestion by an individual to be treated, solid oral dosage forms, aerosols, controlled release formulations, fast melt formulations, effervescent formulations, lyophilized formulations, tablets, powders, pills, dragees, capsules, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations, and mixed immediate release and controlled release formulations. In some embodiments, the pharmaceutical compositions are formulated into capsules. In some embodiments, the pharmaceutical compositions are formulated into solutions (for example, for IV administration). In some cases, the pharmaceutical composition is formulated as an infusion. In some cases, the pharmaceutical composition is formulated as an injection.

[0488] The pharmaceutical solid dosage forms described herein optionally include the presently described fusion protein or a fragment or a variant thereof and one or more pharmaceutically acceptable additives such as a compatible carrier, binder, filling agent, suspending agent, flavoring agent, sweetening agent, disintegrating agent, dispersing agent, surfactant, lubricant, colorant, diluent, solubilizer, moistening agent, plasticizer, stabilizer, penetration enhancer, wetting agent, anti-foaming agent, antioxidant, preservative, or one or more combination thereof.

[0489] In still other aspects, using standard coating procedures, such as those described in Remington's Pharmaceutical Sciences, 20th Edition (2000), a film coating can be provided around the compositions. In some embodiments, the compositions are formulated into particles (for example for administration by capsule) and some or all of the particles can be coated. In some embodiments, the compositions are formulated into particles (for example for administration by capsule) and some or all of the particles can be microencapsulated. In some embodiments, the compositions can be formulated into particles (for example for administration by capsule) and some or all of the particles are not microencapsulated and are uncoated.

[0490] In certain embodiments, compositions provided herein can also include one or more preservatives to inhibit microbial activity. Suitable preservatives include mercury-

containing substances such as merfen and thiomersal; stabilized chlorine dioxide; and quaternary ammonium compounds such as benzalkonium chloride, cetyltrimethylammonium bromide and cetylpyridinium chloride.

[0491] “Antifoaming agents” reduce foaming during processing which can result in coagulation of aqueous dispersions, bubbles in the finished film, or generally impair processing. Exemplary anti-foaming agents include silicon emulsions or sorbitan sesquoleate.

[0492] “Antioxidants” include, for example, butylated hydroxytoluene (BHT), sodium ascorbate, ascorbic acid, sodium metabisulfite and tocopherol. In certain embodiments, antioxidants enhance chemical stability where required.

[0493] Formulations described herein can benefit from antioxidants, metal chelating agents, thiol containing compounds and other general stabilizing agents. Examples of such stabilizing agents, include, but are not limited to: (a) about 0.5% to about 2% w/v glycerol, (b) about 0.1% to about 1% w/v methionine, (c) about 0.1% to about 2% w/v monothioglycerol, (d) about 1 mM to about 10 mM EDTA, (e) about 0.01% to about 2% w/v ascorbic acid, (f) 0.003% to about 0.02% w/v polysorbate 80, (g) 0.001% to about 0.05% w/v. polysorbate 20, (h) arginine, (i) heparin, (j) dextran sulfate, (k) cyclodextrins, (l) pentosan polysulfate and other heparinoids, (m) divalent cations such as magnesium and zinc; or (n) combinations thereof.

[0494] “Binders” impart cohesive qualities and include, *e.g.*, alginic acid and salts thereof; cellulose derivatives such as carboxymethylcellulose, methylcellulose (*e.g.*, Methocel®), hydroxypropylmethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose (*e.g.*, Klucel®), ethylcellulose (*e.g.*, Ethocel®), and microcrystalline cellulose (*e.g.*, Avicel®); microcrystalline dextrose; amylose; magnesium aluminum silicate; polysaccharide acids; bentonites; gelatin; polyvinylpyrrolidone/vinyl acetate copolymer; crospovidone; povidone; starch; pregelatinized starch; tragacanth, dextrin, a sugar, such as sucrose (*e.g.*, Dipac®), glucose, dextrose, molasses, mannitol, sorbitol, xylitol (*e.g.*, Xylitab®), and lactose; a natural or synthetic gum such as acacia, tragacanth, ghatti gum, mucilage of isapol husks, polyvinylpyrrolidone (*e.g.*, Polyvidone® CL, Kollidon® CL, Polyplasdone® XL-10), larch arabogalactan, Veegum®, polyethylene glycol, waxes, sodium alginate, and the like.

[0495] A “carrier” or “carrier materials” include any commonly used excipients in pharmaceuticals and should be selected on the basis of compatibility with compounds disclosed herein, such as, compounds of ibrutinib and an anticancer agent, and the release profile properties of the desired dosage form. Exemplary carrier materials include, *e.g.*,

binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers, stabilizers, lubricants, wetting agents, diluents, and the like.

[0496] “Pharmaceutically compatible carrier materials” can include, but are not limited to, acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, polyvinylpyrrolidone (PVP), cholesterol, cholesterol esters, sodium caseinate, soy lecithin, taurocholic acid, phosphatidylcholine, sodium chloride, tricalcium phosphate, dipotassium phosphate, cellulose and cellulose conjugates, sugars sodium stearoyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, and the like. See, *e.g.*, Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington’s Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980; and Pharmaceutical Dosage Forms and Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins 1999).

[0497] “Dispersing agents,” and/or “viscosity modulating agents” include materials that control the diffusion and homogeneity of a drug through liquid media or a granulation method or blend method. In some embodiments, these agents also facilitate the effectiveness of a coating or eroding matrix. Exemplary diffusion facilitators/dispersing agents include, *e.g.*, hydrophilic polymers, electrolytes, Tween ® 60 or 80, PEG, polyvinylpyrrolidone (PVP; commercially known as Plasdone®), and the carbohydrate-based dispersing agents such as, for example, hydroxypropyl celluloses (*e.g.*, HPC, HPC-SL, and HPC-L), hydroxypropyl methylcelluloses (*e.g.*, HPMC K100, HPMC K4M, HPMC K15M, and HPMC K100M), carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, hydroxypropylmethylcellulose acetate stearate (HPMCAS), noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), vinyl pyrrolidone/vinyl acetate copolymer (S630), 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde (also known as tyloxapol), poloxamers (*e.g.*, Pluronic F68®, F88®, and F108®, which are block copolymers of ethylene oxide and propylene oxide); and poloxamines (*e.g.*, Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (BASF Corporation, Parsippany, N.J.)), polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, polyvinylpyrrolidone/vinyl acetate copolymer (S-630),

polyethylene glycol, *e.g.*, the polyethylene glycol can have a molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, polysorbate-80, sodium alginate, gums, such as, *e.g.*, gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulose, such as, *e.g.*, sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone, carbomers, polyvinyl alcohol (PVA), alginates, chitosans and combinations thereof. Plasticizers such as cellulose or triethyl cellulose can also be used as dispersing agents. Dispersing agents particularly useful in liposomal dispersions and self-emulsifying dispersions are dimyristoyl phosphatidyl choline, natural phosphatidyl choline from eggs, natural phosphatidyl glycerol from eggs, cholesterol and isopropyl myristate.

[0498] Combinations of one or more erosion facilitator with one or more diffusion facilitator can also be used in the present compositions.

[0499] The term “diluent” refers to chemical compounds that are used to dilute the compound of interest prior to delivery. Diluents can also be used to stabilize compounds because they can provide a more stable environment. Salts dissolved in buffered solutions (which also can provide pH control or maintenance) are utilized as diluents in the art, including, but not limited to a phosphate buffered saline solution. In certain embodiments, diluents increase bulk of the composition to facilitate compression or create sufficient bulk for homogenous blend for capsule filling. Such compounds include *e.g.*, lactose, starch, mannitol, sorbitol, dextrose, microcrystalline cellulose such as Avicel®; dibasic calcium phosphate, dicalcium phosphate dihydrate; tricalcium phosphate, calcium phosphate; anhydrous lactose, spray-dried lactose; pregelatinized starch, compressible sugar, such as Di-Pac® (Amstar); mannitol, hydroxypropylmethylcellulose, hydroxypropylmethylcellulose acetate stearate, sucrose-based diluents, confectioner’s sugar; monobasic calcium sulfate monohydrate, calcium sulfate dihydrate; calcium lactate trihydrate, dextrates; hydrolyzed cereal solids, amylose; powdered cellulose, calcium carbonate; glycine, kaolin; mannitol, sodium chloride; inositol, bentonite, and the like.

[0500] “Filling agents” include compounds such as lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose, dextrates, dextran, starches, pregelatinized starch, sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and the like.

- [0501] “Lubricants” and “glidants” are compounds that prevent, reduce or inhibit adhesion or friction of materials. Exemplary lubricants include, *e.g.*, stearic acid, calcium hydroxide, talc, sodium stearyl fumarate, a hydrocarbon such as mineral oil, or hydrogenated vegetable oil such as hydrogenated soybean oil (Sterotex®), higher fatty acids and their alkali-metal and alkaline earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, glycerol, talc, waxes, Stearowet®, boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol (*e.g.*, PEG-4000) or a methoxypolyethylene glycol such as Carbowax™, sodium oleate, sodium benzoate, glyceryl behenate, polyethylene glycol, magnesium or sodium lauryl sulfate, colloidal silica such as Syloid™, Cab-O-Sil®, a starch such as corn starch, silicone oil, a surfactant, and the like.
- [0502] “Plasticizers” are compounds used to soften the microencapsulation material or film coatings to make them less brittle. Suitable plasticizers include, *e.g.*, polyethylene glycols such as PEG 300, PEG 400, PEG 600, PEG 1450, PEG 3350, and PEG 800, stearic acid, propylene glycol, oleic acid, triethyl cellulose and triacetin. In some embodiments, plasticizers can also function as dispersing agents or wetting agents.
- [0503] “Solubilizers” include compounds such as triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, sodium lauryl sulfate, sodium docusate, vitamin E TPGS, dimethylacetamide, N-methylpyrrolidone, N-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropylmethyl cellulose, hydroxypropyl cyclodextrins, ethanol, n-butanol, isopropyl alcohol, cholesterol, bile salts, polyethylene glycol 200-600, glycofurol, transcutool, propylene glycol, and dimethyl isosorbide and the like.
- [0504] “Stabilizers” include compounds such as any antioxidation agents, buffers, acids, preservatives and the like.
- [0505] “Suspending agents” include compounds such as polyvinylpyrrolidone, *e.g.*, polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, vinyl pyrrolidone/vinyl acetate copolymer (S630), polyethylene glycol, *e.g.*, the polyethylene glycol can have a molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, hydroxymethylcellulose acetate stearate, polysorbate-80, hydroxyethylcellulose, sodium alginate, gums, such as, *e.g.*, gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulosics, such as, *e.g.*, sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose,

hydroxyethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone and the like.

[0506] “Surfactants” include compounds such as sodium lauryl sulfate, sodium docusate, Tween 60 or 80, triacetin, vitamin E TPGS, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, copolymers of ethylene oxide and propylene oxide, *e.g.*, Pluronic® (BASF), and the like. Some other surfactants include polyoxyethylene fatty acid glycerides and vegetable oils, *e.g.*, polyoxyethylene (60) hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, *e.g.*, octoxynol 10, octoxynol 40. In some embodiments, surfactants can be included to enhance physical stability or for other purposes.

[0507] “Viscosity enhancing agents” include, *e.g.*, methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, hydroxypropylmethyl cellulose acetate stearate, hydroxypropylmethyl cellulose phthalate, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof.

[0508] “Wetting agents” include compounds such as oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, sodium docusate, sodium oleate, sodium lauryl sulfate, sodium docusate, triacetin, Tween 80, vitamin E TPGS, ammonium salts and the like.

Kits/Article of Manufacture

[0509] Disclosed herein, in certain embodiments, are kits and articles of manufacture for use with one or more methods described herein. Such kits include a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. In one embodiment, the containers are formed from a variety of materials such as glass or plastic.

[0510] The articles of manufacture provided herein contain packaging materials. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, bags, containers, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment.

[0511] A kit typically includes labels listing contents and/or instructions for use, and package inserts with instructions for use. A set of instructions will also typically be included.

[0512] In some embodiments, a label is on or associated with the container. In one embodiment, a label is on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself; a label is associated with a container when it is present within a receptacle or carrier that also holds the container, *e.g.*, as a package insert. In one embodiment, a label is used to indicate that the contents are to be used for a specific therapeutic application. The label also indicates directions for use of the contents, such as in the methods described herein.

EXAMPLES

[0513] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

EXAMPLE 1. Fusion Protein of Anti-PD1-TGF- β Trap

[0514] Anti-PD1 VHs and VLs were synthesized and formatted in IgG, scFv-Fc or scFv configurations with TGF β R2 fused at C-terminal by linker (G4S)₂. Anti-PD1-trap fusion proteins were transiently expressed in Expi293 cells according to manufacturer's protocol. To purify the fusion proteins, the transfected supernatants were loaded to onto a protein L column using AKTA AVANT. The column was washed with PBS, before the protein was eluted with IgG elution buffer (Pierce). The eluted protein was then buffer-exchanged into PBS using a PD-10 column (GE Healthcare).

Surface Plasmon Resonance

[0515] Surface plasma resonance (SPR) analysis was performed using Biacore3000, CM5 chip, an amine-coupling kit, 10X HBS-P running buffer and Glycine for regeneration (GE Healthcare). For anti-PD1 kinetic assay, purified PD-1 Fc fusion protein was immobilized on CM5 chip using a pre-defined ligand immobilization program. Purified anti-PD1-Trap fusion proteins were diluted in HBS-P running buffer to a range of final concentrations and injected. Dissociation was allowed to proceed followed by a pulse of 10 mM Glycine (pH 1.5) for regeneration of the chip surface.

[0516] To investigate if the anti-PD1-trap fusion protein could simultaneously binding to PD1 and TGF β R2, 100 nM of anti-PD1 trap fusion protein was injected to PD1 immobilized CM5 chip for 3 minutes, followed by 3 minutes of dissociation. Subsequently, different isoforms of TGF- β were injected to examine the binding of TGF- β to PD1 bond anti-PD1-trap fusion protein.

[0517] To analyze the binding affinity of anti-PD1-TGF β R2 to TGF- β isoforms, anti-PD1-TGF β R2 were immobilized on CM5 chip and then TGF- β proteins were diluted in HBS-P

running buffer to a range of final concentrations and injected to measure the binding affinity of TGF- β to immobilized anti-PD1-TGF β RII. Affinity of anti-PD1 (VH6-VL5 or VH7-VL6)-TGF β RII fusion protein to immobilized PD-1 is shown in Table 2 (**FIGs. 6-8**).

Affinity of TGF- β isoforms to immobilized anti-PD-1 IgG4 antibody (VH6-VL5)-TGF β RII fusion protein is shown in Table 3. As observed, the fusion protein bound to TGF- β 1 and TGF- β 3 with high affinity and bound to TGF- β 2 with lower affinity.

Table 2. Affinity of anti-PD1-TGF β RII to immobilized PD-1

Anti-PD1-TGF β RII format	K _D (M)
Anti-PD1(VH7-VL6) IgG4-TGF β RII	3.79e-11
Anti-PD1(VH6-VL5) IgG4-TGF β RII	2.95e-11
Anti-PD1(VH7-VL6) IgG1-TGF β RII	8.11e-13
Anti-PD1(VH6-VL5) IgG1-TGF β RII	7.64e-12
Anti-PD1(VH7-VL6) scFv-Fc-TGF β RII	3.53e-10
Anti-PD1(VH6-VL5) scFv-Fc-TGF β RII	2.79e-10
Anti-PD1(VH7-VL6) scFv-TGF β RII	7.77e-9
Anti-PD1(VH6-VL5) scFv-TGF β RII	1.97e-8

Table 3. Affinity of TGF- β to immobilized anti-PD1(VH6-VL5) IgG4-TGF β RII

Anti-PD1-TGF β RII	TGF- β isoform	K _D (M)
Anti-PD1(VH6-VL5) IgG4-TGF β RII	TGF- β 1	5.22e-12
	TGF- β 2	8.59e-10
	TGF- β 3	2.19e-12

[0518] Binding affinity of various anti-PD-1 variants fused to TGF β RII were assessed by surface plasmon resonance (SPR) assay using Biacore 3000. PD-1 fused to human Fc region was immobilized on sensor chip CM5. Different concentrations of various fusion proteins were injected in solution phase and data was analyzed using BIAevaluation to calculate K_D of the fusion proteins. Variants such as anti-PD1(VH6-VL5)-TGF β RII fusion protein and anti-PD1 (VH7-VL6)- TGF β RII fusion protein were found to have similar binding affinity to PD1 compared to respective anti-PD1 alone.

Table 5. Affinity of two anti-PD1-TGF β RII fusion proteins as compared to the respective anti-PD1 antibody alone

Fusion Protein	Binding affinity to PD-1-Fc antigen (K_D , M)
Anti-PD1(VH6-VL5)-TGF β RII	3.42E-13
Anti-PD1(VH6-VL5)	4.74E-13
Anti-PD1(VH7-VL6)-TGF β RII	6.69E-13
Anti-PD1(VH7-VL6)	2.44E-13

PD-1/PDL-1 Blockade Assay

[0519] To investigate if the anti-PD1-TGF β RII fusion proteins could function as anti-PD1 antibody by blocking the PD-1/PD-L1 interaction, a PD-1/PD-L1 blockade bioassay kit from Promega was employed. In brief, the PD-L1 aAPC/CHO-K1 cells were thawed one day prior to the start of the assay and allowed to recover overnight at 37°C, 5% CO₂ for 16 to 20 hours. To generate a dose response curve, serial dilutions of anti-PD1 TGF β RII proteins ranging from 100 nM to 0.098 nM were prepared. The plates were then removed from the incubator and cell media was discarded. Antibodies of interest were then added directly to the plate containing PD-L1 aAPC/CHO-K1 cells. Following this step, PD-1 effector cells were then thawed and added to the 96-well plate. Plates were incubated for at 37°C, 5% CO₂ for 6 hours. Bio-Glo™ luciferase substrate reagent (Promega) was added to each well and Relative Luciferase Units (RLUs) were measured on a Glomax 96-microplate luminometer. The IC₅₀ of the anti-PD1 TGF β RII proteins were determined by fitting the RLU-concentration data to a four parameter logistic equation. These values were used as a measure of PD-1/PD-L1 blocking potency (**FIG. 5**).

HEK-Blue TGF- β Assay

[0520] To examine the efficacy of anti-PD1 TGF β RII fusion proteins to neutralize the function of TGF- β , a stable cell line with induced reporter gene (HEK-Blue™ TGF- β reporter cell line, Invitrogen) was utilized. This stable cell line allows for the detection of bioactive TGF- β via the activation of TGF- β /SMAD pathway, leading to the production of secreted embryonic alkaline phosphatase (SEAP), which can be quantified. In brief, TGF- β 1, TGF- β 2, and TGF- β 3 were diluted to a pre-determined EC₅₀ concentration and added to a flat bottom 96-well plate. To generate a dose response curve, serial dilutions of anti-PD1 TGF β RII proteins were then added to the plate containing different isoforms of TGF- β . Plates were incubated for 30 minutes at 37°C. The HEK-Blue™ TGF- β cells were then

added to the plate at a final concentration of 0.7×10^6 cells/mL. Plates were then incubated at 37°C, 5% CO₂ for a total of 20 hours. Following the incubation, an aliquot of supernatant was removed and added to a plate containing the QUANTI-Blue™ substrate (Invitrogen) used to quantify secreted SEAP. Plates were incubated for one hour at 37°C. The plates were then read on a SpectraMax Plus plate reader at 620 to 655 nm. The IC₅₀ was determined by fitting absorbance-concentration data to a four-parameter logistic equation (GraphPad Prism). Results of the efficacy of various anti-PD1 TGFβRII fusion proteins in comparison to controls are depicted in FIGs. 5-8.

[0521] Table 6 and 7 further lists various fusion protein constructs tested and the inhibition of TGF-β1 induced reported gene activity.

Table 6: Summary of IC₅₀—inhibition of TGF-β1 induced reporter gene activity for various fusion constructs

Fusion Protein	IC ₅₀ (pM)
Anti-PD1 (VL5-VH6).IgG4	9.0
Anti-PD1 (VL5-VH6).IgG4(S108P). TGFbRII	9.7
Anti-PD1 (VL5-VH6) ScFv-Fc	4.3
Anti-PD1 (VL6-VH7).IgG4. TGFbRII	5.6
Anti-PD1 (VL6-VH7).IgG4(S108P). TGFbRII	9.3
Anti-PD1 (nVL1-nVH3).IgG4-TGFbRII	10.7
Anti-PD1 (nVL1-nVH3).scFv-Fc-TGFbRII	6.5
Anti-PD1 (nVL1-nVH7).IgG4-TGFbRII	15.1
Anti-PD1 (nVL1-nVH8).IgG4-TGFbRII	54.5
Anti-RSV IgG4-TGFbRII	8.6
Anti-RSV S108P IgG4-TGFbRII	6.9
Anti-RSV scFv-Fc4	8.9

Table 7: Summary of TGF-β1 neutralization activity of various fusion constructs

Fusion Protein	IC ₅₀ (pM)
Anti-PD1 (VL5/VH6).IgG4-Linker2 - TGFbRII	1.2
Anti-PD1 (VL5/VH6).IgG4-Linker12 - TGFbRII	2.7
Anti-PD1 (VL5/VH6).IgG4-Linker7 - TGFbRII	1.1
Anti-PD1 (VL5/VH6).IgG4-Linker8- TGFbRII	1.2
Anti-PD1 (VL5/VH6).IgG4-Linker10 - TGFbRII	1.9

PD1 binding of various Anti-PD1 IgG4-TGFbRII fusion constructs

[0522] PD1-Fc antigen and reference antigen was immobilized on Biacore CM5 surface. 6-12 replicated series diluted concentrations of various anti-PD1 IgG4-TGFbRII (Table 8) were series injected on PD1-Fc antigen and reference surface. Kinetics data was evaluated for 1:1 model: Langmuir with mass transfer. As observed in Table 7, the binding affinity (K_D) of Anti-PD1 IgG4-TGFbRII with PD1-Fc antigen was between 10¹² to 10¹⁰ range.

Table 8: PD1 binding affinity analysis of PD1 IgG4-TGFbRII fusion proteins

Fusion Protein	K _D (M)
Anti-PD1 (nVL1-nVH3).IgG4	1.48E-11
Anti-PD1 (nVL1-nVH3).IgG4-TGFbRII	2.77E-11
Anti-PD1 (nVL1-nVH3).IgG4(S108P)	1.41E-11
Anti-PD1 (nVL1-nVH3).IgG4-(S108P)-TGFbRII	8.16E-12
Anti-PD1 (nVL1-nVH7).IgG4-TGFbRII	1.47E-10
Anti-PD1 (nVL1-nVH8).IgG4-TGFbRII	3.21E-10

Simultaneous PD1 and TGF-β1 binding of various Anti-PD1 IgG4-TGFβRII fusion constructs

To investigate if the anti-PD1-TGFR^{II} fusion proteins (Table 9) with different linkers could simultaneously bind to PD1 and TGF-β1, the following constructs were made and tested in a Biacore experiment. PD1-Fc antigen was immobilized on a Biacore CM5 chip surface. Anti-PD1 (VL5-VH6).IgG4-linker-TGFbR^{II} was injected over sensor surfaces, followed by TGFβ1 over sensor surfaces. As shown in FIG. 20, all the anti-PD1 (VL5-VH6).IgG4-linker-TGFR^{II} constructs bound to both of PD1-Fc antigen and TGF β1 simultaneously.

Table 9: anti-PD1 (VL5-VH6).IgG4-linker-TGFR^{II} constructs with various linkers

Fusion protein	Linker
Anti-PD1 (VL5-VH6).IgG4-Linker 2 -TGFbR ^{II}	GGGGSGGGS (Linker 2) (SEQ ID NO. 293)
Anti-PD1 (VL5-VH6).IgG4-Linker12 -TGFbR ^{II}	DPVLEREDKVTTSKNPGS (linker 12) (SEQ ID NO: 27)
Anti-PD1 (VL5-VH6).IgG4-Linker7 -TGFbR ^{II}	DPGSGSVPLGSGSNPGS (linker7) (SEQ ID NO: 22)
Anti-PD1 (VL5-VH6).IgG4-Linker8 -TGFbR ^{II}	DPGSGGSVPLGSGGSNPGS (linker 8) (SEQ ID NO: 23)
Anti-PD1 (VL5-VH6).IgG4-Linker10 -TGFbR ^{II}	DPGVLEREDVPTTSYPNPGS (linker10) (SEQ ID NO: 25)

EXAMPLE 2. Anti-PD1-TGF-β Trap promotes T cell Activation in *In vitro* Culture

[0523] To assess the ability of the anti-PD1-TGF-β trap to promote T cells functions, peripheral blood mononuclear cells (PBMCs) purified using Ficoll-Hypaque from a leukapheresis product (obtained from a normal healthy donor under an Institutional Review Board (IRB) approved protocol) was used *in vitro*. PBMCs were labeled with the cell proliferation dye CellTrace violet and stimulated with anti-CD3 and anti-CD28. At the end of the incubation culture supernatants were collected and stored until used for IFN-γ release

assay. The cells were labeled with fluorescent conjugated antibodies to assess T cell proliferation.

[0524] Stimulated PBMCs in the presence of anti-PD1-TGF- β trap showed enhanced proliferation and IFN- γ production in a dose dependent manner compared to anti-PD1 or anti-RSV control antibodies suggesting the ability of the fusion molecule to target both the PD-1/PD-L1 and TGF- β pathways (**FIGS. 9A-9C**). The data also demonstrates an additive enhanced effect of the fusion molecule compared to anti-PD1 alone.

Anti PD1-TGF-RII exhibits superior anti-tumor response compared to anti PD1 alone in an in vitro model of colorectal cancer

[0525] Effectiveness of anti-PD1-TGFR II fusion protein was evaluated in an *in vitro* model of colorectal cancer. PBMCs purified from healthy donor were co-cultured with cancer cell line HT-29 for 5 days. The cells were stimulated with anti-CD3 in the presence of isotype control, anti-PD1 or anti-PD1-TGFR II fusion protein. The culture supernatants were collected and IFN- γ levels were quantified by MSD per manufacturer's protocol. The cells were collected and target occupancy on T cells were analyzed by flow cytometry. The levels of TGF- β 1, TGF- β 2 and TGF- β 3 in cell culture supernatants were quantified by Luminex per manufacturer's protocol.

[0526] **FIG. 9D**, shows that anti-PD1-TGFR II binds to PD1 on CD8⁺ T cell surface similar to that of anti PD1. As shown in **FIG. 9E** anti-PD1-TGFR II fusion protein promoted higher level of IFN- γ production compared to anti-PD1 or isotype control. **FIG. 9H**. TGF- β 1 produced in HT-29 PBMC coculture system is completely neutralized upon anti-PD1-TGFR II treatment even at the lowest dose tested. **FIG. 9I**. TGF- β 2 produced in HT-29 PBMC coculture system is partially neutralized upon anti-PD1-TGFR II treatment. Overall, results from this *in vitro* model exhibit superior anti-tumor response of anti-PD1-TGFR II fusion protein compared to anti-PD1 antibody.

Anti-PD1-TGFR II exhibits superior anti-tumor response compared to anti-PD1 alone in an in vitro model of head and neck cancer

[0527] Effectiveness of anti-PD1-TGFR II fusion protein was evaluated in an *in vitro* model of head and neck cancer. PBMCs purified from healthy donor were co-cultured with cancer cell line Detroit 562 for 5 days. The cells were stimulated with anti-CD3 in the presence of isotype control, anti-PD1 or anti-PD1-TGFR II fusion protein. The culture supernatants were collected and IFN- γ levels were quantified by MSD per manufacturer's protocol. The cells

were collected and target occupancy on T cells were analyzed by flow cytometry. The levels of TGF- β 1, TGF- β 2 and TGF- β 3 in cell culture supernatants were quantified by luminex per manufacturer's protocol.

[0528] **FIG. 9F**, shows that anti-PD1-TGFR2 binds to PD1 on CD8⁺ T cell surface similar to that of anti PD1. As shown in **FIG. 9G**, anti-PD1-TGFR2 fusion protein promoted higher level of IFN- γ production compared to anti-PD1 or isotype control. **FIG. 9J**. TGF- β 1 produced in Detroit 562 PBMC coculture system is completely neutralized upon anti-PD1-TGFR2 treatment even at the lowest dose tested. **FIG. 9K**. TGF- β 2 produced in Detroit 562 PBMC coculture system is partially neutralized upon anti-PD1-TGFR2 treatment. Overall, results from this in vitro model exhibit superior anti-tumor response of anti-PD1-TGFR2 fusion protein compared to anti-PD1 antibody.

Anti PD1-TGF-R2 exhibits superior anti-tumor response compared to anti PD1 alone in an in vitro 3D tumor spheroid model of colorectal cancer

[0529] Effectiveness of anti-PD1-TGFR2 fusion protein was evaluated in an *in vitro* 3D tumor spheroid model of colorectal cancer. 3D tumor spheroids were generated by coculturing colorectal cancer cell line HT-29 and a fibroblast cell line. PBMCs purified from healthy donor were then added to spheroid culture. The cells were stimulated with anti-CD3 in the presence of isotype control, anti-PD1 or anti-PD1-TGFR2 fusion protein for 5 days. The culture supernatants were collected and IFN- γ levels were quantified by MSD per manufacturer's protocol.

[0530] As shown in **FIG. 9L**, anti-PD1-TGFR2 fusion protein promoted higher level of IFN- γ production compared to anti-PD1 or isotype control. Overall, result from this 3D tumor spheroid model exhibit superior anti-tumor response of anti-PD1-TGFR2 fusion protein compared to anti-PD1 antibody.

Anti-PD1-TGF-R2 exhibits superior anti-tumor response compared to anti PD1 alone in an in vitro 3D tumor spheroid model of head and neck cancer

[0531] Effectiveness of anti-PD1-TGFR2 fusion protein was evaluated in an *in vitro* 3D tumor spheroid model of colorectal cancer. 3D tumor spheroids were generated by coculturing Detroit 562 cell line and a fibroblast cell line . PBMCs purified from healthy donor were then added to spheroid culture. The cells were stimulated with anti-CD3 in the presence of isotype control, anti-PD1 or anti-PD1-TGFR2 fusion protein for 5 days. The

culture supernatants were collected and IFN- γ levels were quantified by MSD per manufacturer's protocol.

[0532] As shown in **FIG. 9M**, anti-PD1-TGFR2 fusion protein promoted higher level of IFN- γ production compared to anti-PD1 or isotype control. Overall, result from this 3D tumor spheroid model exhibit superior anti-tumor response of anti-PD1-TGFR2 fusion protein compared to anti-PD1 antibody.

EXAMPLE 3. Anti-PD1-TGF- β Trap has Superior Activity compared to Anti-PD1 in the Presence of Recombinant TGF- β 1 *In vitro*

[0533] To assess the ability of the anti-PD1-TGF- β trap to promote T cells functions, in an environment with high levels of TGF- β , PBMCs were stimulated as above in the presence of recombinant TGF- β 1. At the end of the incubation, culture supernatants were collected and stored until used for cytokine analyses. The cells were labeled with fluorescent conjugated antibodies and assessed for T cell proliferation and the upregulation of IL-2 receptor alpha or CD25 as a readout for activation.

[0534] Recombinant TGF- β 1 suppressed T cell activation, proliferation and cytokine and chemokine production (**FIG. 10 and FIG. 11**). This suppression was reversed significantly by the anti-PD1- TGF- β 1 trap in a dose dependent manner compared to anti-PD1. The data suggests the ability of the fusion protein to neutralize high amount of exogenous TGF- β 1 and disrupt the PD-1/PD-L1 pathway resulting in enhance T cell immune responses. Thus, the blockade of the PD-1/PD-L1 and neutralization of TGF- β by the fusion molecule can be an attractive immunotherapy for eliciting potent anti-tumor responses in cancer indications where checkpoint inhibitors treatments have failed.

EXAMPLE 4. Anti-PD1-TGF-R2 exhibits superior anti-tumor response compared to anti-PD1 alone in a humanized mouse model of colorectal cancer

[0535] Ability of anti-PD1-TGFR2 fusion protein to inhibit colorectal cancer tumor was evaluated in a humanized mouse model. Humanized NOG mice were administered HT-29 cancer cells. Tumor bearing mice were randomized and administered twice weekly with either anti-PD1-TGFR2 fusion protein, anti-PD1 antibody alone or an isotype control. As seen in the FIG.12A, anti-PD1-TGFR2 fusion protein significantly inhibited tumor growth compared to anti-PD1 and the isotype control. Analyses of the tumors at the end of the study showed that anti-PD1-TGFR2 fusion protein treated mice had higher frequency of tumor

infiltrating lymphocytes (TILs). Analysis of tumors from anti-PD1-TGFR2 treated mice also showed higher ratio of CD8⁺ T cells to regulatory T cells (Tregs).

EXAMPLE 5. Anti-PD1-TGFR2 exhibits superior anti-tumor response compared to anti-PD1 alone in an in vitro model of head and neck cancer

[0536] Effectiveness of anti-PD1-TGFR2 fusion protein was evaluated in an in vitro model of head and neck cancer. PBMCs purified from healthy donor were co-cultured with cancer cell line Detroit 562 for 48 hours. The cells were stimulated with anti-CD3 in the presence of isotype control, anti-PD1 or anti-PD1-TGFR2 fusion protein. The culture supernatants were collected and IFN- γ levels were quantified by MSD per manufacturer's protocol. The cell pellets from co-culture at the end of culture period were harvested by centrifugation and RNA was purified using the Qiagen RNeasy micro kit according the manufacturer's protocol. Purified RNA was used for gene expression analysis using the nanostring according to the manufacturer's instructions. Gene expression was analyzed using Nanostring nCounter software.

[0537] As shown in FIG.13A, anti-PD1-TGFR2 fusion protein promoted higher level of IFN- γ production compared to anti-PD1 or isotype control. Anti-PD1-TGFR2 fusion protein treatment resulted in significant upregulation of interferon pathway genes (**FIG. 13B**) as well as cytotoxic genes (**FIG. 13C**) compared to anti-PD1 treatment highlighting improved cytotoxic function in presence of tumor compared to anti-PD1 antibody. Furthermore, anti-PD1-TGFR2 fusion protein treatment resulted in significant downregulation of genes involved in tumor metastasis and angiogenesis pathways (**FIG. 13D**). Overall, results from this in vitro model exhibit superior anti-tumor response of anti-PD1-TGFR2 fusion protein compared to anti-PD1 antibody.

EXAMPLE 6. Anti-PD1-TGFR2 exhibits superior anti-tumor response compared to anti-PD1 alone in a humanized mouse model of head and neck cancer

[0538] Ability of anti-PD1-TGFR2 fusion protein to inhibit head and neck cancer was evaluated in a humanized mouse model. Humanized NSG mice were administered D562 cell line. Tumor bearing mice were randomized in and administered twice weekly with anti-PD1-TGFR2 fusion protein, anti-PD1 antibody alone or an isotype control. As seen in **FIG.14**, Anti-PD1-TGFR2 fusion protein significantly inhibited tumor growth (**FIG.14A**), improved survival of tumor bearing mice (**FIG. 14B**) and increase in IFN γ levels (**FIG. 14F**)

compared to anti-PD1 and the isotype control. Treatment with anti-PD1-TGFR2 fusion protein significantly improved CD8⁺ T cells to Treg ratio in tumors (**FIG. 14C**).

EXAMPLE 7. Anti-PD1-TGFR2 exhibits superior anti-tumor response compared to anti-PD1 alone in primary colorectal cancer (CRC) patient samples

[0539] Effectiveness of anti-PD1-TGFR2 fusion protein was evaluated in primary colorectal cancer patient samples. PBMCs from CRC patients were co-cultured with dissociated tumor cells. The cells were stimulated in the presence of isotype control, anti-PD1 or anti-PD1-TGFR2 fusion protein. The culture supernatants were collected and IFN- γ levels were quantified by MSD per manufacturer's protocol. TGF- β levels were assessed by Luminex per manufacturer's protocol. The cell pellets from co-culture at the end of culture period were harvested by centrifugation and RNA was purified using the Qiagen RNeasy micro kit according to the manufacturer's protocol. Purified RNA was used for gene expression analysis using the nanostring according to the manufacturer's instructions. Gene expression was analyzed using Nanostring nCounter software.

[0540] As shown in **FIG. 15A**, anti-PD1-TGFR2 fusion protein promoted higher level of IFN- γ production compared to anti-PD1 or isotype control. Anti-PD-TGFR2 fusion protein completely neutralized TGF- β 1 produced by patient PBMC and dissociated tumor cells (**FIG. 15B**). Anti-PD1-TGFR2 fusion protein treatment resulted in significant upregulation of cytotoxic genes (**FIG. 15C**) compared to anti-PD1 treatment highlighting improved cytotoxic function in presence of tumor compared to anti-PD1 antibody. Furthermore, anti-PD1-TGFR2 fusion protein treatment resulted in significant downregulation of genes involved in tumor metastasis and angiogenesis pathways (**FIG. 15C**). As expected TGF- β signaling pathway is significantly downregulated in anti-PD1-TGFR2 fusion protein treatment group. Overall, results from this patient derived samples exhibit superior anti-tumor response of anti-PD1-TGFR2 fusion protein compared to anti-PD1 antibody.

EXAMPLE 8. Anti-PD1-TGFR2 exhibits increased cytotoxicity compared to anti-PD-L1-TGFR2 in an *in vitro* model of ovarian cancer

[0541] Effectiveness of Anti- PD1-TGFR2 was compared with Anti-PD-L1-TGFR2 in an *in vitro* model of ovarian cancer. PBMCs purified from healthy donors were co-cultured with cancer cell line SK-OV-3. The immune cells were stimulated in the presence of isotype control, anti-PD1-TGFR2 fusion protein or anti-PD-L1-TGFR2 fusion protein. The killing of tumor cells was assessed across culture period using Incucyte live cell analysis system.

[0542] As shown in **FIG 16**, the presence of anti-PD1-TGFR2 fusion protein exhibited increased killing of tumor cells when compared to anti-PD-L1-TGFR2 fusion protein. Overall, anti-PD1-TGFR2 exhibits improved cytotoxicity when compared to anti-PD-L1-TGFR2.

EXAMPLE 9. Combination of anti-PD1-TGF-R2 with CAR-T significantly enhanced 3D tumor spheroid killing when compared to combination with anti-PD1 or CAR-T alone

[0543] Combination of anti-PD1-TGFR2 fusion protein with CAR-T cells was evaluated in an in vitro 3D tumor spheroid model of ovarian cancer. Note that in this example, CAR-T cells refer to T cells engineered to express MUC16 CAR. 3D tumor spheroids were generated by coculturing ovarian cancer cell line SK-OV-3 and a fibroblast cell line. CAR-T cells were then added to the 3D tumor spheroid culture in the presence of anti-PD1-TGFR2 fusion protein or anti-PD1. The target specific killing of 3D tumor spheroid was assessed across time using Incucyte live cell analysis system.

[0544] As shown in **FIG 17**, the combination of anti-PD1-TGFR2 fusion protein with CAR-T cells showed increased killing of 3-D tumor spheroids when compared to the combination of anti-PD1 and CAR-T cells or CAR-T cells alone. Overall, result from this kill assay suggest that addition of anti-PD-1-TGF-R2 to CAR-T cells enhances the anti-tumor response of CAR-T cells.

EXAMPLE 10 Combination of anti-PD1-TGF-R2 with CD33 specific CAR-T cells

[0545] CD33 CAR-T cells were co-cultured with MOLM-13 AML tumor cell line in a cytotoxicity assay in and the cultures were incubated in the IncuCyte S3 live cell analysis instrument. Anti PD1 or anti-PD1-TGFR2 was added to the indicated cultures at a equimolar concentration. Sytox Green was directly added at the start of the culture to allow for the identification and enumeration of dying cells in the culture. Analysis was performed using the IncuCyte S3 Software. The data presented in the graph shown is the mean $\pm$ SD from triplicate wells, with 5 scans/well taken. As shown in FIG. 18A, the combination of anti-PD1 (VH6/VL5)-TGFR2 fusion protein with the CD33 CAR T cells showed increased killing compared to CAR T cells and anti-PD1(VH6/VL5) combination or CAR T cells alone.

[0546] As shown in FIG. 18B, the combination of anti-PD1(VH7/VL6)-TGFR2 fusion protein with the CD33 CAR-T cells showed increased killing compared to CAR-T cells and anti-PD1 combination or CAR-T cells alone. Overall, the result for these cytotoxicity assays

demonstrate that anti-PD1 (VH6/VL5)-TGFR II fusion protein and anti-PD1(VH7/VL6)-TGFR II fusion protein enhance the anti-tumor immune responses of CAR-T cells.

EXAMPLE 11. Combination of anti-PD1-TGF-R II with CD19 CAR-T cells enhances tumor cell killing

[0547] CD19 CAR-T cells are co-cultured with tumor cells expressing CD19 antigen on cell surface in presence of anti-PD1-TGFR II fusion protein or anti-PD1. The combination of anti-PD1-TGFR II fusion protein with CD19 CAR-T cells shows increase in killing of tumor cells when compared to the combination of anti-PD1 and CAR-T cells or CAR-T cells alone.

EXAMPLE 12. Combination of anti-PD1-TGF-R II with BCMA specific CAR-T improves cytotoxic potential of CAR-T cells

[0548] BCMA specific CAR-T cells are co-cultured with tumor cell line expressing BCMA antigen in presence or absence of anti-PD1-TGFR II fusion protein or anti-PD1. The combination of anti-PD1-TGFR II fusion protein with CAR-T cells improves cytotoxic potential of CAR-T cells compared to the combination of anti-PD1 and CAR-T cells or CAR-T cells alone.

EXAMPLE 13. Combination of anti-PD1-TGF-R II with PSMA specific CAR-T enhances tumor cell killing by CAR-T cells

[0549] PSMA specific CAR-T cells are co-cultured with tumor cell line expressing PSMA antigen in presence or absence of anti-PD1-TGFR II fusion protein or anti-PD1. The combination of anti-PD1-TGFR II fusion protein with CAR-T cells improves cytotoxicity of CAR-T cells when compared to the combination of anti-PD1 and CAR-T cells or CAR-T cells alone.

EXAMPLE 14. NK cell target cell lysis was improved by anti-PD1-TGFR II

[0550] The ability of anti-PD1 (VH7/VL6)-TGFR II and anti-PD1 (VH6/VL5)-TGFR II to promote natural killer cell-mediated killing of tumor cells was assessed in an in vitro model of ovarian cancer. Effector NK cells were purified from healthy donor and co-cultured with SK-OV-3 target cell line overnight at different E:T cell ratios in the presence of anti-RSV-

TFGBR2 (control), anti-PD1 (VH6/VL5) and anti- PD1 (VH7/VL6), anti- PD1 (VH7/VL6)-TGBR2 and anti- PD1 (VH6/VL5)-TGBR2. Propidium iodine was added to the culture to identify dead cells and cell death was assayed using the Nexcelom Celigo. As observed in FIG.19A and FIG.19B, anti-PD1-TGBR2 fusion proteins promoted higher tumor cell killing compared to anti-PD1 or the TGBR2 alone (Anti-RSV-TGBR2).

EXAMPLE 15: Fusion Protein of Anti-PD1-ADA2

[0551] Anti-PD1 VHs and VLs were synthesized and formatted in IgG, scFv-Fc or scFv configurations with adenosine deaminase 2 (ADA2) fused at C-terminal by linker (G4S)2. Anti-PD1- adenosine deaminase fusion proteins were transiently expressed in Expi293 cells according to manufacturer's protocol and purified using AKTA AVANT system.

Surface Plasmon Resonance

[0552] Surface plasma resonance (SPR) analysis was performed using Biacore3000, CM5 chip, an amine-coupling kit, 10X HBS-P running buffer and Glycine for. For anti-PD1 kinetic assay, recombinant PD-1 Fc protein was immobilized on chip using a pre-defined ligand immobilization program. Purified anti-PD1- adenosine deaminase fusion proteins were diluted in running buffer to a range of final concentrations and injected. Dissociation was allowed to proceed followed by regeneration of the chip surface.

Table 10. Affinity of anti-PD1-ADA2 to immobilized PD1

Fusion protein	K _D (M)
Anti-PD1 (VH7-VL6) IgG4-ADA2	6.63e-14
Anti-PD1 (VH6-VL5) IgG4-ADA2	6.16e-14
Anti-PD1 (VH7-VL6) IgG1-ADA2	5.09e-13
Anti-PD1 (VH6-VL5) IgG1-ADA2	3.09e-13
Anti-PD1 (VH7-VL6) scFv-Fc-ADA2	5.63e-10
Anti-PD1 (VH6-VL5) scFv-Fc-ADA2	1.84e-12
Anti-PD1 (VH7-VL6) scFv-ADA2	1.3e-10
Anti-PD1 (VH6-VL5) scFv-ADA2	3.46e-9

PD-1/PDL-1 Blockade Assay

[0553] To investigate if the anti-PD1-ADA2 fusion proteins could function as anti-PD1 antibody by blocking the PD-1/PD-L1 interaction, a PD-1/PD-L1 blockade bioassay kit from

Promega used as depicted in Example 1. These values was used as a measure of PD-1/PD-L1 blocking potency (FIG. 21).

ADA Enzymatic Activity Assay

[0554] To determine the anti-PD1 ADA2 fusion protein enzymatic activity, the colorimetric Adenosine Deaminase (ADA) Activity Kit (Abcam) was utilized according to the manufacturers' recommendations. 2 minutes and 30 minutes timepoints were selected to calculate the ADA enzymatic activity over time. As shown in FIG.22, anti-PD1-ADA2 fusion proteins exhibited enzymatic activity over time.

EXAMPLE 16: PD1 binding affinity analysis of Anti-PD1 IgG4 S108P-wtADA2 and – mutADA2

[0555] PD1-Fc antigen and reference was immobilized on Biacore CM5 surface, then 6-12 replicated series diluted concentrations of Anti-PD1 IgG4 S108P-wtADA2 and -mutADA2 were series injected on PD1-Fc antigen and reference surface. Kinetics data was evaluated for 1:1 model: Langmuir with mass transfer. Results are as depicted in Table 11.

Table 11 Binding affinity of Anti-PD1 (VH6/VL5) and (VH7/VL6) IgG4 S108P and the Anti-PD1-wtADA2 and -mutADA2

Analyte	K _D (M)
Anti-PD1 (VH6/VL5) IgG4 S108P	3.34e-13
Anti-PD1 (VH6/VL5) IgG4 S108P-ADA2	7.53e-13
Anti-PD1 (VH6/VL5) IgG4 S108P-mutADA2	2.18e-13
Anti-PD1 (VH7/VL6) IgG4 S108P	3.92e-13
Anti-PD1 (VH7/VL6) IgG4 S108P-ADA2	4.13e-13
Anti-PD1 (VH7/VL6) IgG4 S108P-mutADA2	3.38e-13

EXAMPLE 17: Anti-PD1-ADA2 Fusion Proteins Effectively Blocked PD-L1/PD1 Signaling

[0556] Ability of anti-PD1-ADA2 fusion proteins to block PD1/PD-L1 interaction was evaluated using a reporter bioassay as mentioned in Example 12.

[0557] As shown in **FIG. 23A-23B**, anti-PD1 (VH7/VL6)-wtADA2 and -mut7ADA2, anti-PD1 (VH6/VL5)-wtADA2 and -mut7ADA2 blocked PD1/PD-L1 interaction with similar potency.

Example 18: ADA2 Enzymatic Activity

[0558] Ability of anti-PD1-ADA2 fusion proteins to enzymatically degrade adenosine was evaluated in an in vitro ADA enzymatic activity assay as mentioned in Example 12.

[0559] As shown in **FIG. 24A-24F**, at lower concentration of Adenosine, mut7ADA2 has higher enzymatic activity compare to the wtADA2.

Example 19: Anti-PD1-mut7-ADA2 has a Higher Enzymatic Activity than Anti-PD1-wtADA2

[0560] The Michaelis Menten constants (K_m) of anti-PD1(VH6/VL5)-wtADA2 and anti-PD1(VH6/VL5)-mut7ADA2 were measured using the fluorometric ADA2 enzyme assay kit. As observed in **FIG. 25**, anti-PD1-mut7ADA2 has a higher K_m compared to anti-PD1-wtADA2.

Example 20: Anti-PD1-wtADA2 Reverses Adenosine-mediated Suppression of T cell Proliferation

[0561] The ability of anti-PD1-wtADA2 to promote T cell proliferation was assessed in vitro using normal donor PBMCs. CellTrace Violet-labeled PBMCs cocultured in media containing 1 mM adenosine were stimulated in the presence of increasing concentrations of anti-PD1(VH6/VL5)-wtADA2, and anti-PD1(VH6/VL5). The supernatants were collected for cytokine analysis. The cells were assayed for T cell proliferation by flow cytometry. The data showed that Anti-PD1 (VH6/VL5)-wtADA2 significantly reversed the adenosine-mediated suppression of $CD4^+$ (**FIG. 26A**) and $CD8^+$ T cells (**FIG. 26B**) compared to Anti-PD1 (VH6/VL5) or isotype control. As observed in **FIG. 26C and D**, both anti-PD1 and anti-PD1-wtADA2 similarly occupied PD1 receptor on $CD4^+$ (**FIG. 26C**) and $CD8^+$ T cells (**FIG. 26D**).

[0562] The ability of anti-PD1(VH7/VL6)-wtADA2 to promote T cells function was further assessed by stimulating PBMCs in media containing adenosine in the presence of increasing concentrations of anti-PD1 (VH7/VL6), anti-PD1(VH7/VL6)-wtADA2 or isotype controls. The culture supernatants were collected and assayed for production of $IFN\gamma$. **FIG. 26E**

shows that anti-PD1(VH7/VL6)-wtADA2 treatment induced significantly more IFN- γ compared to anti-PD1 or isotype control.

Example 21: WtADA2 and mutADA2 Showed Comparable Reversal of Adenosine-mediated Suppression of T cell Proliferation

[0563] The effectiveness of wtADA2 and mutADA2 to reverse adenosine-mediated suppression of T cell proliferation was assessed by stimulating ³H-labeled PBMCs in a media containing adenosine in the presence of increasing concentration of anti-PD1-wtADA2, anti-PD1-mutADA2, anti-RSV-wtADA2 and anti-RSV-mutADA2. The cells were stained, then analyzed for proliferation by flow cytometry. Both the wtADA2 and mutADA fusion proteins were equally effective at reversing the adenosine-mediated suppression of CD4 T cells (**FIG. 27A**) and CD8 T cells (**FIG. 27B**).

Example 22: Blockade of PD1-PDL1 interaction by anti-PD1(VH6/VL5)-ADA2

[0564] Ability of various forms of anti-PD1-ADA2 fusion proteins to block PD1/PD-L1 interaction was evaluated using a reporter bioassay as mentioned in Example 12.

[0565] As shown in **FIG. 28**, anti-PD1 (VH6/VL5) scFv-Fc-ADA2, hIgG1-ADA2 and scFv-ADA2 all showed functional activity as measured by the PD1/PD-L1 Blockade Bioassay.

Example 23: ADA Enzymatic Activity of Anti-PD1-ADA2-scFv-Fc

[0566] Ability of various forms of anti-PD1-ADA2 fusion proteins to enzymatically degrade adenosine was evaluated in an in vitro ADA enzymatic activity assay as mentioned in Example 12.

[0567] As shown in **FIG. 29**, Anti-PD1 (VH7/VL6) scFv-Fc-ADA2 and (VH6/VL5) scFv-Fc-ADA2 have similar ADA2 enzymatic activity comparable to the control.

Example 24: ADA Enzymatic Activity of various anti-PD1-ADA2 and anti-PD1-mutADA2 constructs

[0568] The ADA enzymatic activity assay was conducted as described above. As shown in **FIG. 30**, at 10nM of Adenosine mutADA2 has higher enzymatic activity compared to the wtADA2.

Example 25: anti-PD1-ADA2 Promoted IFN- γ Production and Proliferation of Tumor-infiltrating Lymphocytes (TILs) in Primary CRC Patient Tumor

[0569] PBMCs purified from CRC patients were co-cultured with matched dissociated tumor cells. CellTrace Violet-labeled cells were stimulated with anti-CD3 and anti-CD28 in the presence of isotype control, anti-PD1 (VH6/VL5) or anti-PD1 (VH6/VL5)-wtADA2 fusion protein. The culture supernatants were collected and IFN- γ levels were quantified by MSD per manufacturer's protocol and cells assessed for T cell proliferation. For gene expression analysis, the cells pellets were harvested by centrifugation and RNA was purified using the Qiagen RNeasy micro kit according the manufacturer's protocol. Purified RNA was used for gene expression analysis using the nanostring according to the manufacturer's instructions. Gene expression was analyzed using Nanostring nCounter software.

[0570] As shown in **FIG. 31A**, anti-PD1-wtADA2 fusion protein promoted higher level of IFN- γ production and T cell proliferation (**FIG. 31B**) compared to anti-PD1 or isotype control. anti-PD1-wtADA2 fusion protein treatment resulted in significant upregulation of IFN- γ signaling and chemokine signaling genes and cytotoxic genes (**FIG. 31C**) compared to anti-PD1 treatment highlighting improved cytotoxic function of T cells in the presence of tumor compared to anti-PD1 antibody.

Example 26: Effect of anti-PD1-wtADA2 fusion protein in a humanized mouse model of lung cancer

[0571] NSG mice humanized with peripheral blood mononuclear cells were inoculated intrapancreatically (ortotopic injection) with the pancreatic cancer cell line Panc.08.fLuc.eGFP. Mice body weight were measured weekly and tumor weights were measured at the end of the study on day 38. Mice were randomized into treatment groups and treated twice weekly with HBSS (vehicle control), anti-PD1 (VH6/VL5) and anti-PD1(VH6/VL5) -wtADA2. As can be seen in **FIG. 32**, mice treated with anti-PD-wtADA2 had significantly smaller tumors compared to mice treated with anti-PD1 or isotype control.

[0572] As can be seen in **FIG. 32**, mice treated with anti-PD-wtADA2 had significantly smaller tumors compared to mice treated with anti-PD1 or isotype control.

Example 27: Treatment of Anti-PD1(VH6/VL5)-wtADA2 Downregulated the Adenosine Pathway, angiogenesis and Upregulated Cytotoxicity and Cytokine Genes

[0573] PBMCs purified from CRC patients were co-cultured with matched dissociated tumor cells. CellTrace Violet-labeled cells were stimulated with anti-CD3 and anti-CD28 in the

presence of isotype control, anti-PD1 (VH6/VL5) or anti-PD1 (VH6/VL5)-wtADA2 fusion protein. The culture supernatants were collected and IFN- γ levels were quantified by MSD per manufacturer’s protocol and cells assess for T cell proliferation. For gene expression analysis, the cultules were duplicated and the cells pellets were harvested by centrifugation and RNA was purified using the Qiagen RNAeasy micro kit according the manufacturer’s protocol. Purified RNA was used for gene expression analysis using the nanostring according to the manufacturer’s instructions. Gene expression was analyzed using Nanostring nCounter software.

[0574] Anti-PD1-wtADA2 fusion protein promoted higher level of IFN- γ production and T cell proliferation compared to anti-PD1 or isotype control. Anti-PD1-wtADA2 fusion protein treatment also resulted in significant upregulation of IFN- γ signaling and chemokine signaling genes and cytotoxic genes (**data not shown**) compared to anti-PD1 treatment highlighting improved cytotoxic function of T cells in the presence of tumor compared to anti-PD1 antibody.

SEQUENCES

[0575] Provided herein is a representative list of certain sequences included in embodiments provided herein.

Table 4. Non-limiting exemplary polypeptide and nucleotide sequences

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
Anti-PD1 VH1	1	QVQLVESGGGVVQPGR SLRLSCAASGFTFRSYG MHWVRQAPGKGLEWV AIIFYDGSNKYYADSVK GRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAR DDDYWGQGalVTvss		
Anti-PD1 VH2	2	EVQLVQSGGGVVQPGR SLRLSCAASGFTFSSYG MHWVRQAPGKGLEWV AVIWYDGSNKYYADSV KGRFTISRDNSKNTLYL QMNSLRAEDTAVYYC AGEGFDYWGQGLVT VSS		
Anti-PD1 VH3	3	QVQLVQSGAEVKKPGA SVKVSCKASGYTFTGY YMHWVRQAPGQGLEW MGAINPNSGGTNYAQK FQGRVTMTRDTSISTAY MELSRLRSDDTAVYYC		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		<u>ARRPD</u> RANWHFDYWG QGTLVTVSS		
Anti-PD1 VH4	4	QVQLVQSGAEVKKPG ASVKVSCKASGYTFTG <u>YYMHWVRQAPGQGL</u> EWMGAINPNSGGTNY AQKLQGRVTMTTDS TSTAYMELRSLRSDDT AVYYC <u>ARHGLKGDGY</u> <u>FDYWGQGTLVTVSS</u>		
Anti-PD1 VH5	5	QVQLVESGGGVVQPG RSLRLDCKAS <u>GITFSNS</u> GMHWVRQAPGKGLE WVAVI <u>WYDGSK</u> RYY ADSVKGRFTISRDNK NTLFLQMNSLRAEDTA VYYCAT <u>NNDY</u> WGQG TLVTVSS		
Anti-PD1 VH6	6	QVQLVESGGGVVQPGR SLRLDCKAS <u>GITFSNSG</u> MHWVRQAPGKGLEWV AVI <u>WYDGSK</u> RYYADSV KGRFTISRDNKNTLFL QMNSLRAEDTAVYYC AT <u>NDDY</u> WGQGTLVTV SS	109	caggtgcagctggtcgaaagcggaggaggagtggccagccagg acgateccctgagactggattgtaaggcctctggaatcacattctctaa cagtggaatgcactgggtgcgccaggcaccaggaaaaggactgg agtgggtggccgtcatctggtacgacgggtcaaagcgatactatgc agatagcgtgaaaggaagggtcacaattcacgcgacaacagcaag aatactctgttctgcagatgaactctctgagagcagaggatactgcc gtgtactattgtgctaccaatgacgattattgggggcagggaactctg gtgaccgtcagttca
Anti-PD1 VH7	7	QVQLVQSGVEVKKPGA SVKVSCKASGYTFTNY YMYWVRQAPGQGLEW MGGINPSNGGTNFNEK FKNRVTLTTDSSTTTAY MELKSLQFDDTAVYYC <u>ARRDYRFDMGFDYWG</u> QGTTVTVSS	110	caggtgcagctggccagagcggcgtggaagtcaagaaaccggg ggcctcagtgaaggtcagctgtaaagcttccggctacaccttcacaa actactatatgtattgggtgagacaggcaccaggacagggactgga gtggatggcggggattaaccctagtaatggaggcactaactcaacg aaaagttaaaaacagggtgaccctgaccacagattcaagcactacc acagcttacatggagctgaagtcctgcagtttgacgatacagccgt gtactattgtgctcggagagactacagggttcgatatgggctttgactat tggggccaggggactaccgtgaccgtctcctct
Anti-PD1 VL1	8	EIVMTQSPATLSLSPGE RATLSCR <u>ASQSVSSYLA</u> <u>WYQQKPGQAPRLLIYD</u> ASNRATGIPARFSGSGS GTDFTLTISLEPEDFAV YYC <u>QQYNNWPRTFGQ</u> GTKVEIK		
Anti-PD1 VL2	9	EIVLTQSPATLSLSPGER ATLSCR <u>ASQSVSSYLA</u> <u>WYQQKPGQAPRLLIYD</u> ASNRATGIPARFSGSGS GTDFTLTISLEPEDFAV YYC <u>QQRSNWPKTFGQG</u> TKVEIK		
Anti-PD1 VL3	10	RNVLTQSPLSLPVTSGE PASISCRSS <u>QSLSSSGYT</u> <u>YLDWYLQKPGQSPQLL</u> IY <u>LASWRDSGVPDRFSG</u> SGSGTDFTLKISRVEAE DVGVYYC <u>MQAEQTPG</u> <u>PGNTEGQGTKLEIK</u>		
Anti-PD1 VL4	11	DVVMTQSPLSLPVTSGE PASISCRSS <u>QSL</u> LHTNG		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		<u>YNYLHWYLQKPGQSPQ</u> <u>LLIYLGSWQDSGVPDRF</u> SGSGSGTDFTLKISRVE AEDVGVYYCMQAEQT <u>PRTFGQGTRLEVK</u>		
Anti-PD1 VL5	12	EIVLTQSPATLSLSPGER ATLSCRASQSVSSYLA WYQQKPGQAPRLLIYD <u>ASNRATGIPARFSGSGS</u> GTDFTLTISSLEPEDFAV YYCQSSNWPRTFGQG TKVEIK	111	 gagatcgtcctgacacagagtcagcaactctgagcctgtccccg gcgaacgagctactctgtcctgccgggcatctcagagtgtgtctagtt acctggcctggatcagcagaagccccggccaggctcctaggctgct gatctacgacgccagcaacagagctaccgggattcctgccaggttct caggcagcgggtccggaacagactttaccctgacaatctcaagcct ggagcccgaagatttcgctgtgtactattgccagcagtcctctaattg gcctcgcacctttggccaggggacaaaaggcgcgagatcaag
Anti-PD1 VL6	13	EIVLTQSPATLSLSPGER ATLSCRASKGVSTSGYS <u>YLHWYQQKPGQAPRL</u> <u>IYLASYLESGVPARFSG</u> SGSGTDFTLTISSLEPED FAVYYCQHSRDLPLTF GGGTKVEIK	112	 gagatcgtcctgactcagtcacccagcaaccctgagtctgtcaccagg agaaagggcaaccctgagctgccgagcatccaagggggtgagca catccggatactcttatctgcactgggtaccagcagaaacccggacag gtcctcgactgctgatctacctggcatcttatctggagagtggcgtg cctgctcggttctctgggagtggatcaggcaccgattttacactgact atttctagctggagccagaagatttcgcagtgtactattgccagcatt ctcgagacctgccctgacatttggcgggggaactaaggcgcgagat caaa
TGFβRII ECD	14	IPPHVQKSVNNDMIVTD NNGAVKFPQLCKFCDV RFSTCDNQKSCMSNCSI TSICEKPQEVCAVWR KNDENITLETVCHDPKL PYHDFILED AASPKCIM KEKKKPGETFFMCSCSS DECNDNIIFSEEYNTSNP D		
TGFβRII2 ECD	141	IPPHVQKSDVEMEAQK DEIICPSCNRTAHPLRHI NNDMIVTDNNGAVKFP QLCKFCDVRFSTCDNQ KSCMSNCSITSICEKPQE VCVAVWRKNDENITLE TVCHDPKL PYHDFILED AASPKCIMKEKKKPGE TFFMCSCSSDECNDNIIF SEEYNTSNPD		
TGFβRII min ECD	142	PQLCKFCDVRFSTCDN QKSCMSNCSITSICEKP QEVCAVWRKNDENIT LETVCHDPKL PYHDFIL EDAASPKCIMKEKKKP GETFFMCSCSSDECND NIIFSEEYNTSNPD		
TGFβRII	289	MGRGLLRGLWPLHIVL WTRIASTIPPHVQKSDV EMEAQKDEIICPSCNRT AHPLRHINNDMIVTDN NGAVKFPQLCKFCDVR FSTCDNQKSCMSNCSIT SICEKPQEVCAVWRK NDENITLETVCHDPKL PYHDFILED AASPKCIMK EKKKPGETFFMCSCSSD ECNDNIIFSEEYNTSNPD		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		LLLVIFQVTGISLLPPLG VAISVIIIIFYCYRVNRQQ KLSSTWETGKTRKLME FSEHCAIILEDDRSDISS TCANNINHNTPELL DTLVGKGRFAEVYKAK LKQNTSEQFETVAVKIF PYEEYASWKTEKDIFSD INLKHENILQFLTAEER KTELGKQYWLITAFHA KGNLQEYLTRHVISWE DLRKLGSSLARGIAHLH SDHTPCGRPKMPIVHR DLKSSNILVKNDLTCCL CDFGLSLRLDPTLSVDD LANSGQVGTARYMAPE VLESRMNLENVESFKQ TDVYSMALVLWEMTS RCNAVGEVKDYEPFG SKVREHPCVESMKDNV LRDRGRPEIPFWLNHQ GIQMCETLTECWDHD PEARLTAQCVAERFSEL EHLDRLSGRSCSEKIP EDGSLNTTK		
TGFβRII Isoform B	290	MGRGLLRGLWPLHIVL WTRIASTIPPHVQKSVN NDMIVTDNNGAVKFPQ LCKFCDVRFSTCDNQK SCMSNCSITSICEKPQEV CVAVWRKNDENITLET VCHDPKLPYHFILEDA ASPKCIMKEKKKPGETF FMCSCSSDECNDNIIFSE EYNTSNPDLLL VIFQVT GISLLPPLGVAISVIIIIFY CYRVNRQQKLSSTWET GKTRKLMEFSEHCAIIL EDDRSDISSTCANNINH NTELLPIELDTLVGKGR FAEVYKAKLKQNTSEQ FETVAVKIFPYEEYASW KTEKDIFSDINLKHENIL QFLTAEERKTELGKQY WLITAFHAKGNLQEYL TRHVISWEDLRKLGSSL ARGIAHLHSDHTPCGRP KMPIVHRDLKSSNILVK NDLTCCLCDFGLSLRLD PTLSVDDLANSQVGT ARYMAPEVLESRMNLE NVESFKQTDVYSMALV LWEMTSRCNAVGEVK DYEPFGSKVREHPCVE SMKDNVLRDRGRPEIPS FWLNHQGIQMCETLT ECWDHDPEARLTAQCV		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		AERFSELEHLDRLSGRS CSEEKIPEDGSLNTTK		
Anti- PD1(VL5) IgG4- (light chain)	15	EIVLTQSPATLSLSPGERAT LSCRASQSVSSYLAWYQKP GQAPRLLIYD ASNRATGIPARFSGSGSGTD FTLTISSLEPEDFAVYYCQQ SSNWPRTFGQ GTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFY PREAKVQWKV DNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHK VYACEVTHQG LSSPVTKSFNRGEC	299	GAGATCGTCCTGACACAGAGTCCAGCAACTCTGAGC CTGTCCCCCGGCGAACGAGCTACTCTGTCTGCCGG GCATCTCAGAGTGTGTCTAGTTACCTGGCCTGGTAT CAGCAGAAGCCCGGCCAGGCTCCTAGGCTGCTGATC TACGACGCCAGCAACAGAGCTACCGGGATTCTGCC AGGTTCTCAGGCAGCGGGTCCGGAACAGACTTTACC CTGACAATCTCAAGCCTGGAGCCCGAAGATTTTCGCT GTGTACTATTGCCAGCAGTCCTCTAATTGGCCTCGC ACCTTTGGCCAGGGGACAAAGGTCGAGATCAAGCGT ACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCA TCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTT GTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCC AAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCG GGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGC AAGGACAGCACCTACAGCCTCAGCAGCACCTGACG CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTAC GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCC GTCACAAAGAGCTTCAACAGGGGAGAGTGT
Anti- PD1(VL6) IgG4- (light chain)	296	EIVLTQSPATLSLSPGERAT LSCRASKGVSTSGYSYLHWY QQKPGQAPRL LIYLASYLESQVGPARGSG SGTDFTLTISSLEPEDFAVY YCQHSRDLPL TFGGGKVEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLL NNFYPPREAKV QWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADY EKHKVYACEV THQGLSSPVTKSFNRGEC	300	GAGATCGTCCTGACTCAGTCCCCAGCAACCCTGAGT CTGTCACCAGGAGAAAGGGCAACCCTGAGCTGCCGA GCATCCAAGGGGGTGAGCACATCCGGATACTCTTAT CTGCACTGGTACCAGCAGAAACCCGGACAGGCTCCT CGACTGCTGATCTACCTGGCATCTTATCTGGAGAGT GGCGTGCCTGCTCGGTTCTCTGGGAGTGGATCAGGC ACCGATTTTACACTGACTATTTCTAGTCTGGAGCCA GAAGATTTTCGAGTGTACTATTGCCAGCATTCTCGA GACCTGCCCCTGACATTTGGCGGGGGAAGTAAAGGTC GAGATCAAACGTACGGTGGCTGCACCATCTGTCTTC ATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA ACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTAT CCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACACA GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGC AGCACCTGACGCTGAGCAAAGCAGACTACGAGAAA CACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGC CTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGA GAGTGT
Anti-PD1 (VH6) IgG4- linker- TGFβRII	16	QVQLVESGGGVVQPGR SLRLDCKASGITFSNSG MHWVRQAPGKGLEWV AVIWYDGSKRYYADSV KGRFTISRDNSTLFL QMNSLRAEDTAVYYC ATNDDYWQGTLVTV SSASTKGPSVFPLAPCS RSTSESTAALGCLVKD YFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSL SSVVTVPSSSLGTKTYT CNVDHKPSNTKVDKRV ESKYGPPCPSCPAPEFL GGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSQE	113	CAGGTGCAGCTGGTCGAAAGCGGAGGAG GAGTGGTCCAGCCAGGACGATCCCTGAGA CTGGATTGTAAGGCCTCTGGAATCACATT CTCTAACAGTGGAATGCACTGGGTGCGCC AGGCACCAGGAAAAGGACTGGAGTGGGT GGCCGTCATCTGGTACGACGGGTCAAAGC GATACTATGCAGATAGCGTGAAAGGAAG GTTTCAACAATTTACGCGACAACAGCAAGA ATACTCTGTTTCTGCAGATGAACTCTCTGA GAGCAGAGGATACTGCCGTGTACTATTGT GCTACCAATGACGATTATTGGGGGCAGGG AACTCTGGTGACCGTCAGTTCAGCTAGCA CCAAGGGCCCATCGGTCTTCCCCCTGGCG CCCTGCTCCAGGAGCACCTCCGAGAGCAC AGCCGCCCTGGGCTGCCTGGTCAAGGACT ACTTCCCCGAACCGGTGACGGTGTCTGTGG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		DPEVQFNWYVDGVEV HNAKTKPREEQFNSTY RVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVY TLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWES NGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEAL HNHYTQKSLSLSPGKG GGSGGGGSIPPHVQKS VNNDMIVTDNNGAVKF PQLCKFCDVRFSTCDN QKSCMSNCSITSICEKP QEVCVAVWRKNDENIT LETVCHDPKLPYHDFIL EDAASPKCIMKEKKKP GETFFMCSCSSDECND NIIFSEEYNTSNPD		AACTCAGGCGCCCTGACCAGCGGCGTGCA CACCTTCCCGGCTGTCCTACAGTCCTCAG GACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGAC CTACACCTGCAACGTAGATCACAAGCCCA GCAACACCAAGGTGGACAAGAGAGTTGA GTCCAAATATGGTCCCCCATGCCCATCAT GCCCAGCACCTGAGTTCCTGGGGGGACCA TCAGTCTTCCTGTTCCCCCAAAACCCAA GGACACTCTCATGATCTCCCGGACCCCTG AGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTG GTACGTGGATGGCGTGGAGGTGCATAATG CCAAGACAAAGCCGCGGGAGGAGCAGTT CAACAGCACGTACCGTGTGGTCAGCGTCC TCACCGTCCTGCACCAGGACTGGCTGAAC GGCAAGGAGTACAAGTGCAAGGTCTCCA ACAAAGGCCTCCCGTCCTCCATCGAGAAA ACCATCTCCAAAGCCAAAGGGCAGCCCCG AGAGCCACAGGTGTACACCCTGCCCCCAT CCCAGGAGGAGATGACCAAGAACCAGGT CAGCCTGACCTGCCTGGTCAAAGGCTTCT ACCCCAGCGACATCGCCGTGGAGTGGGA GAGCAATGGGCAGCCGGAGAACAACACTAC AAGACCACGCCTCCCGTGCTGGACTCCGA CGGCTCCTTCTTCTCTACAGCAGGCTCAC CGTGGACAAGAGCAGGTGGCAGGAGGGG AATGTCTTCTCATGCTCCGTGATGCATGA GGCTCTGCACAACCACTACACACAGAAGA GCCTCTCCCTGTCTCCGGGTAAAGGTGGA GGTGGTTCTGGAGGTGGAGGTAGTATCCC TCCTCACGTACAGAAGTCCGTGAACAATG ACATGATTGTCACTGACAATAACGGAGCC GTCAAGTTTCTCAGCTATGTAAGTTCTGC GATGTTTCGGTTCTCCACATGCGATAATCA GAAAAGCTGTATGTCTAATTGCAGTATCA CTAGTATATGCGAAAAACCTCAAGAAGTT TGCGTCGCCGTGTGGCGGAAAAATGATGA AAATATCACGCTTGAGACTGTCTGCCATG ATCCAAAGTTACCCTACCACGACTTCATC TTAGAAGACGCCGCATCACCCAAGTGCAT TATGAAAGAGAAAAAGAAGCCAGGAGAA ACATTCTTTATGTGCTCATGCTCCTCTGAC GAATGCAACGACAACATTATCTTCTCTGA GGAGTATAACACCTCAAATCCAGAC
Anti-PD1 (VH6) IgG4- (S108P). linker- TGFβRII	143	QVQLVESGGGVVQPGR SLRLDCKASGITFSNSG MHWVRQAPGKGLEWV AV IWYDGSKRYYADSVKG RFTISRDN SKNTLFLQM NSLRAEDTAVYYCATN D DYWGQGT LVTVSSAST KGPSVFPLAPCSRSTSES TAALGCLVKDYFPEPV TVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVT	114	CAGGTGCAGCTGGTCGAAAGCGGAGGAG GAGTGGTCCAGCCAGGACGATCCCTGAGA CTGGATTGTAAGGCCTCTGGAATCACATT CTCTAACAGTGGAATGCACTGGGTGCGCC AGGCACCAGGAAAAGGACTGGAGTGGGT GGCCGTCATCTGGTACGACGGGTCAAAGC GATACTATGCAGATAGCGTGAAAGGAAG GTTTACAATTTACGCGACAACAGCAAGA ATACTCTGTTTCTGCAGATGAACTCTCTGA GAGCAGAGGATACTGCCGTGTACTATTGT GCTACCAATGACGATTATTGGGGGCAGGG AACTCTGGTGACCGTCAGTTCAGCTAGCA CCAAGGGCCCATCGGTCTTCCCCCTGGCG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		VPSSSLGTKTYTCNV DH KPSNTKVDKRVESKY GPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRT PEVETCVVVDVSQEDPE VQFNWYVDGVEVHNAK TKPREEQFNSTYRVVSV LT VLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFY PSDIAVEWESNGQPEN NYKTTTPVLDSDSGFFL Y SRLTVDKSRWQEGNVF SCSVMHEALHNHYTQK SLSLSPGKGGGSGGG GS IPPHVQKSVNNDMIVTD NNGAVKFPQLCKFCDV RFSTCDNQKSCMSNCSI TSICEKPQEVCVAVWR KNDENITLETVCHDPKL PYHDFILEDAAAPKCI MKEKKKPGETFFMCSS SDECNDNIIFSEEYNTS NP D		CCCTGCTCCAGGAGCACCTCCGAGAGCAC AGCCGCCCTGGGCTGCCTGGTCAAGGACT ACTTCCCCGAACCGGTGACGGTGTCTGTGG AACTCAGGCGCCCTGACCAGCGGCGTGCA CACCTTCCCGGCTGTCTACAGTCCTCAG GACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGAC CTACACCTGCAACGTAGATCACAAGCCCA GCAACACCAAGGTGGACAAGAGAGTTGA GTCCAAATATGGTCCCCCATGCCACCAT GCCCAGCACCTGAGTTCCTGGGGGGACCA TCAGTCTTCCTGTTCCCCCCAAAACCCAA GGACACTCTCATGATCTCCCGGACCCCTG AGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTG GTACGTGGATGGCGTGGAGGTGCATAATG CCAAGACAAAGCCGCGGGAGGAGCAGTT CAACAGCACGTACCGTGTGGTCAGCGTCC TCACCGTCCTGCACCAGGACTGGCTGAAC GGCAAGGAGTACAAGTGAAGGTCTCCA ACAAAGGCCTCCCGTCCTCCATCGAGAAA ACCATCTCCAAAGCCAAAGGGCAGCCCCG AGAGCCACAGGTGTACACCCTGCCCCCAT CCCAGGAGGAGATGACCAAGAACCAGGT CAGCCTGACCTGCCTGGTCAAAGGCTTCT ACCCAGCGACATCGCCGTGGAGTGGGA GAGCAATGGGCAGCCGGAGAACAACTAC AAGACCACGCCTCCCGTGCTGGACTCCGA CGGCTCCTTCTTCTCTACAGCAGGCTCAC CGTGGACAAGAGCAGGTGGCAGGAGGGG AATGTCTTCTCATGCTCCGTGATGCATGA GGCTCTGCACAACCACTACACACAGAAGA GCCTCTCCCTGTCTCCGGGTAAAGGTGGA GGTGGTTCTGGAGGTGGAGGTAGTATCCC TCCTCACGTACAGAAGTCCGTGAACAATG ACATGATTGTCACTGACAATAACGGAGCC GTCAAGTTTCTCCTCAGCTATGTAAGTTCTGC GATGTTCCGGTTCTCCACATGCGATAATCA GAAAAGCTGTATGTCTAATTGCAGTATCA CTAGTATATGCGAAAAACCTCAAGAAGTT TGCGTCGCCGTGTGGCGGAAAAATGATGA AAATATCACGCTTGAGACTGTCTGCCATG ATCCAAAGTTACCCTACCACGACTTCATC TTAGAAGACGCCGCATCACCCAAGTGCAT TATGAAAGAGAAAAAGAAGCCAGGAGAA ACATTCTTTATGTGCTCATGCTCCTCTGAC GAATGCAACGACAACATTATCTTCTCTGA GGAGTATAACACCTCAAATCCAGAC
Anti-PD1 VH7 IgG4 (S108P) linker- TGFbRII	144	QVQLVQSGVEVKKPGA SVKVSCKASGYTFTNY YMYWVRQAPGQGLEW MGG INPSNGGTNFNEKFKNR VTLTDSSTTTAYMEL KSLQFDDTAVYYCARR D YRFDMGFDYWQGQTT VTVSSASTKGPSVFPLA	115	CAGGTGCAGCTGGTCCAGAGCGGCGTGG AAGTCAAGAAACCCGGGGCCTCAGTGAA GGTCAGCTGTAAAGCTTCCGGCTACACCT TCACAACTACTATATGTATTGGGTGAGA CAGGCACCAGGACAGGGACTGGAGTGGA TGGGCGGGATTAACCCTAGTAATGGAGGC ACTAACTTCAACGAAAAGTTTAAAAACAG GGTGACCCTGACCACAGATTCAAGCACTA CCACAGCTTACATGGAGCTGAAGTCCCTG CAGTTTGACGATACAGCCGTGTACTATTG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		PCSRSTSESTAALGCLV K DYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKT YTCNVDPHKPSNTKV DK RVESKYGPPCPPCPAPE FLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVS QEDPEVQFNWYVDGV EVHNAKTKPREEQFNS TY RVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVY T LPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWES NGQPENNYKTTPPVLD S DGSFFLYSRLTVDKSR WQEGNVFSCSVMHEAL HNHYTQKSLSLSPGKG GG GSGGGGSIPPHVQKSVN NDMIVTDNNGAVKFPQ LCKFCDVRFSTCDNQK S CMSNCSITSICEKPQEV CVAVWRKNDENITLET VCHDPKLPYHDFILED A ASPKCIMKEKKKPGETF FMCSSSDECNDNIIFSE EYNTSNPD*		TGCTCGGAGAGACTACAGGTTTCGATATGG GCTTTGACTATTGGGGCCAGGGGACTACC GTGACCGTCTCCTCTGCTAGCACCAAGGG CCCATCGGTCTTCCCCCTGGCGCCCTGCTC CAGGAGCACCTCCGAGAGCACAGCCGCC CTGGGCTGCCTGGTCAAGGACTACTTCCC CGAACCGGTGACGGTGTCTGGAAGTCTCAG GCGCCCTGACCAGCGGCGTGCACACCTTC CCGGCTGTCTTACAGTCCTCAGGACTCTA CTCCCTCAGCAGCGTGGTGACCGTGCCCT CCAGCAGCTTGGGCACGAAGACCTACACC TGCAACGTAGATCACAAGCCCAGCAACAC CAAGGTGGACAAGAGAGTTGAGTCCAAA TATGGTCCCCCATGCCCACCATGCCCAGC ACCTGAGTTCCTGGGGGGACCATCAGTCT TCCTGTTCCCCCCTAAAACCCAAGGACACT CTCATGATCTCCCGGACCCCTGAGGTCAC GTGCGTGGTGGTGGACGTGAGCCAGGAA GACCCCGAGGTCCAGTTCAACTGGTACGT GGATGGCGTGGAGGTGCATAATGCCAAG ACAAAGCCGCGGGAGGAGCAGTTCAACA GCACGTACCGTGTGGTCAGCGTCCTCACC GTCCTGCACCAGGACTGGCTGAACGGCAA GGAGTACAAGTGCAAGGTCTCCAACAAA GGCCTCCCGTCTCTCCATCGAGAAAACCAT CTCAAAGCCAAAGGGCAGCCCCGAGAG CCACAGGTGTACACCCTGCCCCCATCCCA GGAGGAGATGACCAAGAACCAGGTCAGC CTGACCTGCCTGGTCAAAGGCTTCTACCC CAGCGACATCGCCGTGGAGTGGGAGAGC AATGGGCAGCCGGAGAACAACTACAAGA CCACGCCTCCCGTGTCTGGACTCCGACGGC TCCTTCTTCTCTACAGCAGGCTCACCGTG GACAAGAGCAGGTGGCAGGAGGGGAATG TCTTCTCATGCTCCGTGATGCATGAGGCTC TGCACAACCACTACACACAGAAGAGCCTC TCCCTGTCTCCGGGTAAAGGTGGAGGTGG TTCTGGAGGTGGAGGTAGTATCCCTCCTC ACGTACAGAAGTCCGTGAACAATGACATG ATTGTCAGTACATAACGGAGCCGTCAA GTTTCCTCAGCTATGTAAGTTCTGCGATGT TCGGTTCTCCACATGCGATAATCAGAAAA GCTGTATGTCTAATTGCAGTATCACTAGT ATATGCGAAAAACCTCAAGAAGTTTGCGT CGCCGTGTGGCGGAAAAATGATGAAAAT ATCACGCTTGAGACTGTCTGCCATGATCC AAAGTTACCCTACCACGACTTCATCTTAG AAGACGCCGCATCACCCAAGTGCATTATG AAAGAGAAAAAGAAGCCAGGAGAAACAT TCTTTATGTGCTCATGCTCCTCTGACGAAT GCAACGACAACATTATCTTCTCTGAGGAG TATAACACCTCAAATCCAGAC
Anti-PD1 VH7 IgG4 (linker- TGFbRII	145	QVQLVQSGVEVKKPGA SVKVSCKASGYTFTNY YMYWVRQAPGQGLEW MGG INPSNGGTNFNEKFKNR VTLTDSSTTTAYMEL	116	CAGGTGCAGCTGGTCCAGAGCGGCGTGG AAGTCAAGAAACCCGGGGCCTCAGTGAA GGTCAGCTGTAAAGCTTCCGGCTACACCT TCACAACTACTATATGTATTGGGTGAGA CAGGCACCAGGACAGGGACTGGAGTGGA TGGGCGGGATTAACCCTAGTAATGGAGGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		KSLQFDDTAVYYCARR D YRFDMGFDYWGQGTT VTVSSASTKGPSVFPLA PCSRSTSESTAALGCLV K DYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKT YTCNVDPHKPSNTKVDK RVESKYGPPCPSCPAPE FLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVS QEDPEVQFNWYVDGV EVHNAKTKPREEQFNS TY RVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVY T LPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWES NGQPENNYKTTPPVLD S DGSFFLYSRLTVDKSR WQEGNVFSCSVMHEAL HNHYTQKSLSLSPGKG GG GSGGGGSIPPHVQKSVN NDMIVTDNNGAVKFPQ LCKFCDVRFSTCDNQK S CMSNCSITSICEKPQEV CVAVWRKNDENITLET VCHDPKLPYHDFILED A ASPKCIMKEKKKPGETF FMCSCSSDECNDNIIFSE EYNTSNPD		ACTAACTTCAACGAAAAGTTTAAAAACAG GGTGACCCTGACCACAGATTCAAGCACTA CCACAGCTTACATGGAGCTGAAGTCCCTG CAGTTTGACGATACAGCCGTGTACTATTG TGCTCGGAGAGACTACAGGTTTCGATATGG GCTTTGACTATTGGGGCCAGGGGACTACC GTGACCGTCTCCTCTGCTAGCACCAAGGG CCCATCGGTCTTCCCCCTGGCGCCCTGCTC CAGGAGCACCTCCGAGAGCACAGCCGCC CTGGGCTGCCTGGTCAAGGACTACTTCCC CGAACCGGTGACGGTGTCGTGGAACCTCAG GCGCCCTGACCAGCGGCGTGCACACCTTC CCGGCTGTCCTACAGTCCTCAGGACTCTA CTCCCTCAGCAGCGTGGTGACCGTGCCCT CCAGCAGCTTGGGCACGAAGACCTACACC TGCAACGTAGATCACAAGCCCAGCAACAC CAAGGTGGACAAGAGAGTTGAGTCCAAA TATGGTCCCCCATGCCCATCATGCCCAGC ACCTGAGTTCCTGGGGGGACCATCAGTCT TCCTGTTCCCCCCTAAAACCCAAGGACACT CTCATGATCTCCCGGACCCCTGAGGTCAC GTGCGTGGTGGTGGACGTGAGCCAGGAA GACCCCGAGGTCCAGTTCAACTGGTACGT GGATGGCGTGGAGGTGCATAATGCCAAG ACAAAGCCGCGGGAGGAGCAGTTCAACA GCACGTACCGTGTGGTCAGCGTCCTCACC GTCCTGCACCAGGACTGGCTGAACGGCAA GGAGTACAAGTGCAAGGTCTCCAACAAA GGCCTCCCGTCCCTCCATCGAGAAAACCAT CTCAAAGCCAAAGGGCAGCCCCGAGAG CCACAGGTGTACACCCTGCCCCCATCCCA GGAGGAGATGACCAAGAACCAGGTCAGC CTGACCTGCCTGGTCAAAGGCTTCTACCC CAGCGACATCGCCGTGGAGTGGGAGAGC AATGGGCAGCCGGAGAACAACTACAAGA CCACGCCTCCCGTGCTGGACTCCGACGGC TCCTTCTTCTCTACAGCAGGCTCACCGTG GACAAGAGCAGGTGGCAGGAGGGGAATG TCTTCTCATGCTCCGTGATGCATGAGGCTC TGCACAACCACTACACACAGAAGAGCCTC TCCCTGTCTCCGGGTAAAGGTGGAGGTGG TTCTGGAGGTGGAGGTAGTATCCCTCCTC ACGTACAGAAGTCCGTGAACAATGACATG ATTGTCACTGACAATAACGGAGCCGTCAA GTTTCCTCAGCTATGTAAGTTCTGCGATGT TCGGTTCTCCACATGCGATAATCAGAAAA GCTGTATGTCTAATTGCAGTATCACTAGT ATATGCGAAAAACCTCAAGAAGTTTGCGT CGCCGTGTGGCGGAAAAATGATGAAAAT ATCACGCTTGAGACTGTCTGCCATGATCC AAAGTTACCCTACCACGACTTCATCTTAG AAGACGCCGCATCACCAAGTGCATTATG AAAGAGAAAAAGAAGCCAGGAGAAACAT TCTTTATGTGCTCATGCTCCTCTGACGAAT GCAACGACAACATTATCTTCTCTGAGGAG TATAACACCTCAAATCCAGAC
VH6-	294	QVQLVESGGGVVQPGR SLRLDCKASGITFSNSG	297	CAGGTGCAGCTGGTCGAAAGCGGAGGAG GAGTGGTCCAGCCAGGACGATCCCTGAGA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
IgG4(mut) -linker- ECD		MHWVRQAPGKGLEWV AV IWYDGSKRYYADSVKG RFTISRDNSKNTLFLQM NSLRAEDTAVYYCATN D DYWGQGTLVTVSSAST KGPSVFPLAPCSRSTSES TAALGCLVKDYFPEPV TVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVT VPSSSLGTKTYTCNVD H KPSNTKVDKRVESKYG PPCPPCPAPEFLGGPSVF LFPPKPKDTLMISRT EVTCTVVDVSQEDPEV QFNWYVDGVEVHNAK TKPREEQFNSTYRVVSV LT VLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFY PSDIAVEWESNGQPEN NYKTTTPVLDSGDSFFL Y SRLTVDKSRWQEGNVF SCSVMHEALHNHYTQK SLSLSLGKGGGSGGG GS IPPHVQKSVNNDMIVTD NNGAVKFPQLCKFCDV RFSTCDNQKSCMSNCSI TSICEKPQEVCAVWR KNDENITLETVCHDPKL PYHDFILEDAAAPKCI MKEKKKPGETFFMCSS DECNDNIIFSEEYNTSNP D*		CTGGATTGTAAGGCCTCTGGAATCACATT CTCTAACAGTGGAATGCACTGGGTGCGCC AGGCACCAGGAAAAGGACTGGAGTGGGT GGCCGTCATCTGGTACGACGGGTCAAAGC GATACTATGCAGATAGCGTGAAAGGAAG GTTCAACAATTTACGCGACAACAGCAAGA ATACTCTGTTTCTGCAGATGAACTCTCTGA GAGCAGAGGATACTGCCGTGTACTATTGT GCTACCAATGACGATTATTGGGGGCAGGG AACTCTGGTGACCGTCAGTTCAGCTAGCA CCAAGGGCCCATCGGTCTTCCCCCTGGCG CCCTGCTCCAGGAGCACCTCCGAGAGCAC AGCCGCCCTGGGCTGCCTGGTCAAGGACT ACTTCCCCGAACCGGTGACGGTGTCTGTGG AACTCAGGCGCCCTGACCAGCGGCGTGCA CACCTTCCCGGCTGTCCTACAGTCCTCAG GACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACGAAGAC CTACACCTGCAACGTAGATCACAAGCCCA GCAACACCAAGGTGGACAAGAGAGTTGA GTCCAAATATGGTCCCCCATGCCACCAT GCCCAGCACCTGAGTTCCTGGGGGGACCA TCAGTCTTCCTGTTCCCCCCAAAACCCAA GGACACTCTCATGATCTCCCGGACCCCTG AGGTCACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTG GTACGTGGATGGCGTGGAGGTGCATAATG CCAAGACAAAGCCGCGGGAGGAGCAGTT CAACAGCACGTACCGTGTGGTCAGCGTCC TCACCGTCCTGCACCAGGACTGGCTGAAC GGCAAGGAGTACAAGTGCAAGGTCTCCA ACAAAGGCCTCCCGTCCTCCATCGAGAAA ACCATCTCCAAAGCCAAAGGGCAGCCCCG AGAGCCACAGGTGTACACCCTGCCCCCAT CCCAGGAGGAGATGACCAAGAACCAGGT CAGCCTGACCTGCCTGGTCAAAGGCTTCT ACCCACGCGACATCGCCGTGGAGTGGGA GAGCAATGGGCAGCCGGAGAACAACTAC AAGACCACGCCTCCCGTGCTGGACTCCGA CGGCTCCTTCTTCTCTACAGCAGGCTCAC CGTGGACAAGAGCAGGTGGCAGGAGGGG AATGTCTTCTCATGCTCCGTGATGCATGA GGCTCTGCACAACCACTACACACAGAAGA GCCTCTCCCTGTCTCTGGGTAAAGGTGGA GGTGGTTCTGGAGGTGGAGGTAGTATCCC TCCTCACGTACAGAAGTCCGTGAACAATG ACATGATTGTCACTGACAATAACGGAGCC GTCAAGTTTCCTCAGCTATGTAAGTTCTGC GATGTTCCGGTTCTCCACATGCGATAATCA GAAAAGCTGTATGTCTAATTGCAGTATCA CTAGTATATGCGAAAAACCTCAAGAAGTT TGCGTCGCCGTGTGGCGGAAAAATGATGA AAATATCACGCTTGAGACTGTCTGCCATG ATCCAAAGTTACCCTACCACGACTTCATC TTAGAAGACGCCGCATCACCCAAGTGCAT TATGAAAGAGAAAAAGAAGCCAGGAGAA ACATTCTTTATGTGCTCATGCTCCTCTGAC GAATGCAACGACAACATTATCTTCTCTGA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GGAGTATAACACCTCAAATCCAGAC
VH7-IgG4(mut)-linker-ECD	295	QVQLVQSGVEVKKPGA SVKVSCKASGYTFTNY YMYWVRQAPGQGLEW MGG INPSNGGTNFNEKFKNR VTLTDSSTTTAYMEL KSLQFDDTAVYYCARR D YRFDMGFDYWQGTT VTVSSASTKGPSVFPLA PCSRSTSESTAALGCLV K DYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDK RVESKYGPPCPPCPAPE FLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVS QEDPEVQFNWYVDGV EVHNAKTKPREEQFNS TY RVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVY T LPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWES NGQPENNYKTPPVLD S DGSFFLYSRLTVDKSR WQEGNVFSCSVMHEAL HNHYTQKSLSLGLKGG GG GSGGGGSIPPHVQKSVN NDMIVTDNNGAVKFPQ LCKFCDVRFSTCDNQK S CMSNCSITSICEKPQEV CVAVWRKNDENITLET VCHDPKLPYHDFILED ASPKCIMKEKKKPGETF FMCSCSSDECNDNIIFSE EYNTSNPD*	298	CAGGTGCAGCTGGTCCAGAGCGGCGTGG AAGTCAAGAAACCCGGGGCCTCAGTGAA GGTCAGCTGTAAAGCTTCCGGCTACACCT TCACAACTACTATATGTATTGGGTGAGA CAGGCACCAGGACAGGGACTGGAGTGGA TGGGCGGGATTAACCCTAGTAATGGAGGC ACTAACTTCAACGAAAAGTTTAAAAACAG GGTGACCCTGACCACAGATTCAAGCACTA CCACAGCTTACATGGAGCTGAAGTCCCTG CAGTTTGACGATACAGCCGTGTACTATTG TGCTCGGAGAGACTACAGGTTTCGATATGG GCTTTGACTATTGGGGCCAGGGGACTACC GTGACCGTCTCCTCTGCTAGCACCAAGGG CCCATCGGTCTTCCCCCTGGCGCCCTGCTC CAGGAGCACCTCCGAGAGCACAGCCGCC CTGGGCTGCCTGGTCAAGGACTACTTCCC CGAACCGGTGACGGTGTCTGGAAGTCTCAG GCGCCCTGACCAGCGGCGTGCACACCTTC CCGGCTGTCTACAGTCCTCAGGACTCTA CTCCCTCAGCAGCGTGGTGACCGTGCCCT CCAGCAGCTTGGGCACGAAGACCTACACC TGCAACGTAGATCACAAGCCCAGCAACAC CAAGGTGGACAAGAGAGTTGAGTCCAAA TATGGTCCCCCATGCCCACCATGCCCAGC ACCTGAGTTCCTGGGGGGACCATCAGTCT TCCTGTTCCCCCCTAAACCAAGGACACT CTCATGATCTCCCGGACCCCTGAGGTCAC GTGCGTGGTGGTGGACGTGAGCCAGGAA GACCCCGAGGTCCAGTTCAACTGGTACGT GGATGGCGTGGAGGTGCATAATGCCAAG ACAAAGCCGCGGGAGGAGCAGTTCAACA GCACGTACCGTGTGGTCAGCGTCCTCACC GTCCTGCACCAGGACTGGCTGAACGGCAA GGAGTACAAGTGCAAGGTCTCCAACAAA GGCCTCCCGTCTCTCCATCGAGAAAACCAT CTCAAAGCCAAAGGGCAGCCCCGAGAG CCACAGGTGTACACCCTGCCCCCATCCCA GGAGGAGATGACCAAGAACCAGGTCAGC CTGACCTGCCTGGTCAAAGGCTTCTACCC CAGCGACATCGCCGTGGAGTGGGAGAGC AATGGGCAGCCGGAGAACAACTACAAGA CCACGCCTCCCGTGTCTGGACTCCGACGGC TCCTTCTTCTCTACAGCAGGCTCACCGTG GACAAGAGCAGGTGGCAGGAGGGGAATG TCTTCTCATGCTCCGTGATGCATGAGGCTC TGCACAACCACTACACACAGAAGAGCCTC TCCCTGTCTCTGGGTAAAGGTGGAGGTGG TTCTGGAGGTGGAGGTAGTATCCCTCCTC ACGTACAGAAGTCCGTGAACAATGACATG ATTGTCAGTACATAACGGAGCCGTCAA GTTTCCTCAGCTATGTAAGTTCTGCGATGT TCGGTTCTCCACATGCGATAATCAGAAAA GCTGTATGTCTAATTGCAGTATCACTAGT ATATGCGAAAAACCTCAAGAAGTTTGCGT CGCCGTGTGGCGGAAAAATGATGAAAAT ATCACGCTTGAGACTGTCTGCCATGATCC AAAGTTACCCTACCACGACTTCATCTTAG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				AAGACGCCGCATCACCCAAGTGCATTATG AAAGAGAAAAAGAAGCCAGGAGAAACAT TCTTTATGTGCTCATGCTCCTCTGACGAAT GCAACGACAACATTATCTTCTCTGAGGAG TATAACACCTCAAATCCAGAC
Linker	17	DPGGGGSGGGGSNPGS		
Linker	18	GGGGSGGGGSGBP		
Linker	19	DPGSGGGSGGGSGS		
Linker	20	GGGGSGGGSGGGGSD PGS		
Linker	21	DPGSGGGSGGGSGG GGS		
Linker 7	22	DPGSGSVPLGSGSNPGS		
Linker 8	23	DPGSGSVPLGSGSNP GS		
Linker	24	DPGVLEREDKPTTSKPN PGS		
Linker 10	25	DPGVLEREDVPTTSYPN PGS		
Linker	26	DPGVLEREDKVTTSKY NPGS		
Linker 12	27	DPVLEREDKVTTSKNP GS		
Linker	28	DIEGRMD		
Linker	29	GEGKSSGSGSESKAS		
Linker	30	GSTSGSGKPGSGEGSTK G		
Linker	31	A(EAAAK) ₄ ALEA(EAAA K) ₄ A		
Linker	32	(G4S) _n , n=1-10		
Linker	33	(Gly) _n , n=6-8		
Linker	34	(EAAAK) _n , n=1-6		
hM195 VL	35	DIQMTQSPSSLSASVGD RVTITCRASESDNYGI SFMNWFQKPGKAPKL LIYAASNQSGVPSRFS GSGSGTDFTLTISSLQPD DFATYYCQQSKEVPWT FGQGTVKVEIK	72	gacattcagatgacccagtcctcgagctctctgctccgcatcagtagg agacagggtcaccatcacatgcagagccagcgaaagtgtcgacaa ttatggcattagctttatgaactggtccaacagaaacccgggaaggc tctaagcttctgattacgctgcacccaaccaaggtccgggtacc ctctcgcttctcaggcagtgatctgggacagacttactctcaccatt tcattctgcagcctgatgacttcgaacctattactgtcagcaagta aggaggttccgtggacgttcggtcaagggaccaaggtggagatca aa
hM195 VH	36	QVQLVQSGAEVKKPGS SVKVSCKASGYTFTDY NMHWVRQAPGQGLEW IGYIYPYNGGTGYNQKF KSKATITADESTNTAY MELSSLRSEDYAVYYC ARGRPAMDYWGQGT LTVSS	73	caggttcagctggtgcagctctggagctgaggtgaagaagcctggga gctcagtgaaaggttctgcaaagcttctggctacaccttactgacta caacatgcactgggtgaggcaggctcctggccaaggcctggaatg gattggatattttatccttacaatggtggtaccggctacaaccagaag ttcaagagcaaggccacaattacagcagacgagagtactaacacag cctacatggaactctccagcctgaggtctgaggacactgcagcttatt actgcgcaagagggcgccccgctatggactactggggccaaggg actctggtcactgtctctca
M2H12 VH	37	QVQLQQSGPELVRPGT FVKISCKASGYTFTNYD INWVNQRPGQGLEWIG WIYPGDGSTKYNEKF KAKATLTADKSSSTAY LQLNNLTSENSAVYFC ASGYEDAMDYWGQGT SVTVSS		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
M2H12 VL	38	DIKMTQSPSSMYASLGE RVIINCKASQDINSYLS WFQQKPGKSPKTLIYR ANRLVDGVPSRFSGSG SGQDYSLTISSLEYEDM GIYYCLQYDEFPLTFGA GTKLELKR		
DRB2 VH	39	EVKLQESGPELVKPGAS VKMSCKASGYKFTDYV VHWLKQKPGQGLEWI GYINPYNDGTKYNEKF KGKATLTSDKSSSTAY MEVSSLTSEDSAVYYC ARDYRYEVYGM DYWG QGTSVTVSS		
DRB2 VL	40	DIVLTQSPTIMSASPGER VTMTCTASSSVNYIH WYQQKSGDSPLRWIFD TSKVASGVPARFSGSG SGTSYSLTISTMEAEDA ATYYCQQWRSYPLTFG DGRLELKRADAAPT VS		
My9-6 VH	41	QVQLQQPGA EVVKPGA SVKMSCKASGYTFTS YYIHWIKQTPGQGLEW VGVIYPGNDDISYNQK FKGKATLTADKSSTTA YMQLSLTSEDSAVYY CAREVRLRYFDVWGA GTTVTVSS		
My9-6 VL	42	NIMLTQSPSSLA VSAGE KVTMSCKSSQS VFFSS SQKNYLAWYQQIPGQS PKLLIYWASTRESGVP DRFTGSGSGTDFTLT ISSVQSEDLAIYYCHQ YLS SRTFGGGTKLEIKR		
MUC16-1 VL	43	DIELTQSPSSLA VSAGE KVTMSCKSSQSLLNSRT RKNQLAWYQQKPGQS PELLIYWASTRQSGVPD RFTGSGSGTDFTLT ISSVQAEDLAVYYCQ QSYNL LTFGPGTKLEV KR	74	GACATCGAGCTGACACAGAGCCCATCTAG CCTGGCTGTGTCTGCCGGCGAGAAAGTGA CCATGAGCTGCAAGAGCAGCCAGAGCCT GCTGAACAGCCGGACCAGAAAGAATCAG CTGGCCTGGTATCAGCAGAAGCCCGGCCA ATCTCCTGAGCTGCTGATCTACTGGGCCA GCACAAGACAGAGCGGCGTGCCCGATAG ATTCACAGGATCTGGCAGCGGCACCGACT TCACCCTGACAATCAGTTCTGTGCAGGCC GAGGACCTGGCCGTGTACTACTGTCAGCA GAGCTACAACCTGCTGACCTTCGGACCCG GCACCAAGCTGGAAGTGAAGAGA
MUC16-1 VH	44	VKLQESGGGFVKPGGS LKVSCAASGFTFSSYA MSWVRLSPEMRLEWV ATISSAGGYIFYSDSVQ GRFTISRDN AKNTLHLQ MGSLSRSGDTAMYYCA RQGF GNYGDYYAMDY WGQGTTVTVSS	75	GTGAAGCTGCAAGAGTCCGGCGGAGGCTT TGTGAAGCCTGGCGGCTCTCTGAAAGTGT CCTGTGCCGCCAGCGGCTTCACCTTTAGC AGCTACGCCATGAGCTGGGTCCGACTGAG CCCTGAGATGAGACTGGAATGGGTGCGCA CCATCAGTAGCGCAGGCGGCTACATCTTC TACAGCGACTCTGTGCAGGGCAGATTAC CATCAGCCGGGACAACGCCAAGAACACC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CTGCACCTCCAGATGGGCAGTCTGAGAAG CGGCGATACCGCCATGTACTACTGCGCCA GACAAGGCTTCGGCAACTACGGCGACTAC TATGCCATGGATTACTGGGGCCAGGGCAC CACCGTGACAGTCTCTTCT
MUC16-2 VL	45	DIELTQSPSSLAVSAGE KVTMSCKSSQSLLNSRT RKNQLAWYQQKTGQS PELLIYWASTRQSGVPD RFTGSGSGTDFTLTISV QAEDLAVYYCQQSYNL LTFGPGTKLEIKR	76	GACATCGAGCTGACACAGAGCCCATCTAG CCTGGCTGTGTCTGCCGGCGAGAAAGTGA CCATGAGCTGCAAGAGCAGCCAGAGCCT GCTGAACAGCCGGACCAGAAAGAATCAG CTGGCCTGGTATCAGCAGAAAACCGGACA GAGCCCCGAGCTGCTGATCTACTGGGCCA GCACAAGACAGAGCGGCGTGCCCGATAG ATTCACAGGATCTGGCAGCGGCACCGACT TCACCCTGACAATCAGTTCTGTGCAGGCC GAGGACCTGGCCGTGTACTACTGTCAGCA GAGCTACAACCTGCTGACCTTCGGACCCG GCACCAAGCTGGAAATCAAGAGA
MUC16-2 VH	46	VKLEESGGGFVKPGGS LKISCAASGFTFRNYAM SWVRLSPEMRLEWVAT ISSAGGYIFYSDSVQGR FTISRDNANKNTHLQMG SLRSGDTAMYYCARQG FGNYGDYYAMDYWGQ GTTVTVSS	77	GTGAAGCTGGAAGAGTCCGGCGGAGGCT TTGTGAAGCCTGGCGGAAGCCTGAAGATC AGCTGTGCCGCCAGCGGCTTCACCTTCAG AAACTACGCCATGAGCTGGGTCCGACTGA GCCCCGAGATGAGACTGGAATGGGTGCGC ACAATCAGCAGCGCAGGCGGCTACATCTT CTACAGCGATAGCGTGCAGGGCAGATTCA CCATCAGCCGGGACAACGCCAAGAACAC CCTGCACCTCCAGATGGGCAGTCTGAGAT CTGGCGACACCGCCATGTACTACTGCGCC AGACAAGGCTTCGGCAACTACGGCGACTA CTATGCCATGGATTACTGGGGCCAGGGCA CCACCGTGACAGTCTCTTCT
MUC16-3 VL	47	DIKMAQSPSSVNASLGE RVTITCKASRDINNLS WFHQKPGKSPKTLIYR ANRLVDGVPSRFSGSGS GQDYSFTISSLEYEDVG IYYCLQYGDLYTFGGG TKLEIK	78	GACATCAAGATGGCTCAGTCCCCTTCTAG CGTGAATGCTTCGCTAGGGGAGCGTGTGA CCATCACATGTAAAGCATCACGCGACATA AATAATTTCCCTTTCCTGGTTTCATCAGAAA CCGGGCAAGTCGCCTAAGACGCTGATTTA CAGAGCAAATCGGTTGGTAGATGGAGTGC CAAGCAGATTACAGCGGGAGCGGAAGTGG ACAGGATTATAGCTTCACTATTTTCATCCCT GGAATACGAGGACGTAGGTATCTATTATT GCCTCCAGTATGGCGATCTTTACACATTT GGTGGGGGGGACTAAGCTGGAGATTAAG
MUC16-3 VH	48	DVQLLES GPGLVRPSQS LSLTCSVTGYSIVSHYY WNWIRQFPGNKLEWM GYISSDGSNEYNP SLKN RISISLDTSKNQFFLKFD FVTTADTATYFCVRGV DYWGQGTTLTVSS	79	GACGTGCAACTTCTGGAGAGCGGGCCAG GGCTAGTCAGGCCCTCCCAGTCGCTTTCA CTGACTTGCAGTGTGACCGGTTACTCTATT GTGAGTCACTACTATTGGAAGTGGATTTCG GCAGTTCCCAGGCAACAACTGGAATGG ATGGGGTACATATCTTCCGATGGCTCGAA TGAATATAACCCATCATTGAAAAATCGTA TTTCCATCAGTCTGGATACGAGTAAAAAC CAGTTTTTTCCTCAAATTTCGATTTCTGACT ACAGCAGATACTGCCACATACTTCTGTGT ACGAGGTGTGCGATTATTGGGGACAGGGCA CAACGCTGACCGTAAGTTCT
MUC16-4 VL	49	DIQMTQSSSFLSVSLGG RVTITCKASDLIHNWLA WYQQKPGNAPRLLISG ATSLETGVPSRFSGSGS GNDYTLSIASLQTEDAA	80	GACATCCAGATGACCCAGAGCAGCAGCTT CCTGAGCGTGTCCCTTGGCGGCAGAGTGA CCATCACCTGTAAAGCCAGCGACCTGATC CACAACCTGGCTGGCCTGGTATCAGCAGAA GCCTGGCAACGCTCCCAGACTGCTGATTA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		TYYCQQYWTTTPFTFGS GTKLEIK		GCGGCGCCACCTCTCTGGAAACAGGCGTG CCAAGCAGATTTTCCGGCAGCGGCTCCGG CAACGACTACACACTGTCTATTGCCAGCC TGCAGACCGAGGATGCCGCCACCTATTAC TGCCAGCAGTACTGGACCACACCTTTCAC CTTTGGCAGCGGCACCAAGCTGGAAATCA AG
MUC16-4 VH	50	DVQLQESGPGLVNPSQ SLSLTCTVTGYSITNDY AWNWRQFPGNKLEW MGYINYSGYTTYNPSL KSRISITRDTSKNQFFLH LNSVTTEDTATYYCAR WDGGLTYWGQGTLVT VSA	81	GACGTTTCAGCTGCAAGAGTCTGGCCCTGG CCTGGTCAATCCTAGCCAGAGCCTGAGCC TGACATGTACCGTGACCGGCTACAGCATC ACCAACGACTACGCCTGGAAGTGGATCAG ACAGTTCCCCGGCAACAAGCTGGAATGGA TGGGCTACATCAACTACAGCGGCTACACC ACCTACAATCCCAGCCTGAAGTCCCGGAT CTCCATCACCAGAGACACCAGCAAGAACC AGTTCTTCCTGCACCTGAACAGCGTGACC ACCGAGGATACCGCCACCTACTACTGCGC TAGATGGGATGGCGGCCTGACATATTGGG GCCAGGGAACACTGGTCACCGTGTCTGCT
MUC16-5 VL	51	DIQMTQSPSSLSASVGD RVTITCKASDLIHNWLA WYQQKPGKAPKLLISG ATSLETGVPSRFSGSGS GTDFTLTISSLQPEDFAT YYCQQYWTTTPFTFGQG TKVEIKR	82	GACATCCAGATGACCCAGAGCCCCAGCA GCCTGAGCGCCAGCGTGGGCGACAGGGT GACCATCACCTGCAAGGCCAGCGACCTGA TCCACAACCTGGCTGGCCTGGTACCAGCAG AAGCCCGGCAAGGCCCCCAAGCTGCTGAT CAGCGGCGCCACCAGCCTGGAGACCGGC GTGCCCAGCAGGTTTCAGCGGCAGCGGCA GCGGCACCGACTTCACCCTGACCATCAGC AGCCTGCAGCCCGAGGACTTCGCCACCTA CTACTGCCAGCAGTACTGGACCACCCCT TCACCTTCGGCCAGGGCACCAAGGTGGAG ATCAAGAGG
MUC16-5 VH-L	52	EVQLVESGGGLVQPGG SLRLSCAASGYSTNDY AWNWRQAPGKGLEW VGYINYSGYTTYNPSLK SRFTISRDN SKNTLYLQ MNSLRAEDTAVYYCAR WDGGLTYWGQGTLVT VSS	83	GAGGTGCAGCTGGTGGAGAGCGGCGGCG GCCTGGTGCAGCCCGGCGGCAGCCTGAGG CTGAGCTGCGCCGCCAGCGGCTACAGCAT CACCAACGACTACGCCTGGAAGTGGGTGA GGCAGGCCCCCGGCAAGGGCCTGGAGTG GGTGGGCTACATCAACTACAGCGGCTACA CCACCTACAACCCAGCCTGAAGAGCAGG TTCACCATCAGCAGGGACAACAGCAAGA ACACCCTGTACCTGCAGATGAACAGCCTG AGGGCCGAGGACACCGCCGTGTACTACTG CGCCAGGTGGGACGGCGGCCTGACCTACT GGGGCCAGGGCACCTGGTGACCGTGAG CAGC
MUC16-5 VH-F	53	EVQLVESGGGLVQPGG SLRLSCAASGYSTNDY AWNWRQAPGKGLEW VGYINYSGYTTYNPSLK SRFTISRDN SKNTFYLQ MNSLRAEDTAVYYCAR WDGGLTYWGQGTLVT VSS	84	GAGGTGCAGCTGGTGGAGAGCGGCGGCG GCCTGGTGCAGCCCGGCGGCAGCCTGAGG CTGAGCTGCGCCGCCAGCGGCTACAGCAT CACCAACGACTACGCCTGGAAGTGGGTGA GGCAGGCCCCCGGCAAGGGCCTGGAGTG GGTGGGCTACATCAACTACAGCGGCTACA CCACCTACAACCCAGCCTGAAGAGCAGG TTCACCATCAGCAGGGACAACAGCAAGA ACACCTTCTACCTGCAGATGAACAGCCTG AGGGCCGAGGACACCGCCGTGTACTACTG CGCCAGGTGGGACGGCGGCCTGACCTACT GGGGCCAGGGCACCTGGTGACCGTGAG CAGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
MUC16-6 VL	54	DIVLTQSPAIMSASLGE RVTMTCTASSSVSSSYL HWYQQKPGSSPKLWIY STSNLASGVPGRFSGSG SGTSYSLTISSMEAEDA ATYYCHQYHRSPYTFG GGTKVEIKR	85	GACATCGTGCTGACACAGAGCCCTGCCAT CATGTCTGCCAGCCTCGGCGAGCGAGTGA CCATGACATGTACAGCCAGCAGCAGCGTG TCCAGCAGCTACCTGCATTGGTATCAGCA GAAGCCCCGGCAGCAGCCCCAAGCTGTGG ATCTACAGCACAAGCAATCTGGCCAGCGG CGTGCCAGGCAGATTTTCTGGTTCTGGCA GCGGCACCAGCTACAGCCTGACAATCAGC AGCATGGAAGCCGAGGATGCCGCCACCT ACTACTGCCACCAGTACCACAGAAGCCCC TACACCTTTGGCGGAGGCACCAAGGTGGA AATCAAGCGG
MUC16-7 VL	55	DIQMTQSPSSLSASVGD RVTITCTASSSVSSSYLH WYQQKPGKAPKLLIYS TSNLASGVPSRFSGSGS GTDFTLTISLQPEDFAT YYCHQYHRSPYTFGQG TKVEIKR	86	GACATCCAGATGACACAGAGCCCTAGCA GCCTGTCTGCCAGCGTGGGAGACAGAGTG ACCATCACCTGTACAGCCAGCAGCAGCGT GTCCAGCAGCTACCTGCATTGGTATCAGC AGAAGCCCCGGCAAGGCCCTAAGCTGCTG ATCTACAGCACCAGCAATCTGGCCAGCGG CGTGCCAAGCAGATTTTCTGGCTCTGGCA GCGGCACCGACTTCACCCTGACCATATCT AGCCTGCAGCCTGAGGACTTCGCCACCTA CTACTGCCACCAGTACCACAGAAGCCCCT ACACCTTTGGCCAGGGCACCAAGGTGGAA ATCAAGCGG
MUC16-7 VH	56	EVQLVESGGGLVQPGG SLRLSCAASGFNIKDTY MHWVRQAPGKGLEWV GRVDPANGNTKYDPKF QGRFTISADTSKNTAYL QMNSLRAEDTAVYYC VRDYYGHTYGFAPWG QGTLVTVSS	87	GAGGTGCAGCTGGTTGAATCTGGCGGAGG ACTGGTTCAGCCTGGCGGATCTCTGAGAC TGTCTTGTGCCGCCAGCGGCTTCAACATC AAGGACACCTACATGCACTGGGTCCGACA GGCCCCCTGGCAAAGGACTTGAGTGGGTTG GAAGAGTGGACCCCGCCAACGGCAACAC CAAATACGACCCCAAGTTCAGGGCAGAT TCACCATCAGCGCCGACACCAGCAAGAAC ACCGCCTACCTGCAGATGAACAGCCTGAG AGCCGAGGACACCGCCGTGTACTATTGCG TGCGGGATTACTACGGCCATACCTACGGC TTCGCCTTTTGGGGCCAGGGCACACTGGT TACCGTTAGCTCT
CD8alpha hinge	57	KPTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGG AVHTRGLDFACD	88	AAGCCCACCACCACCCCTGCCCCCTAGACC TCCAACCCAGCCCCCTACAATCGCCAGCC AGCCCCTGAGCCTGAGGCCCGAAGCCTGT AGACCTGCCGCTGGCGGAGCCGTGCACAC CAGAGGCCTGGATTTTCGCCTGCGAC
CD8alpha 2x	58	KPTTTPAPRPPTPAPTIA SQPLSLRPEASRPAAGG AVHTRGLDFASDKPTT TPAPRPPTPAPTIASQPL SLRPEACRPAAGGAVH TRGLDFACD	89	AAACCTACTACAACCTCCTGCCCCCGGCC TCCTACACCAGCTCCTACTATCGCCTCCCA GCCACTCAGTCTCAGACCCGAGGCTTCTA GGCCAGCGGCCGGAGGCGCGGTCCACAC CCGCGGGCTGGACTTTGCATCCGATAAGC CCACCACCACCCCTGCCCCCTAGACCTCCA ACCCAGCCCCCTACAATCGCCAGCCAGCC CCTGAGCCTGAGGCCCGAAGCCTGTAGAC CTGCCGCTGGCGGAGCCGTGCACACCAGA GGCCTGGATTTTCGCCTGCGAC
CD8alpha 3x	59	KPTTTPAPRPPTPAPTIA SQPLSLRPEASRPAAGG AVHTRGLDFASDKPTT TPAPRPPTPAPTIASQPL SLRPEASRPAAGGAVH TRGLDFASDKPTTTPAP	90	AAGCCTACCACCACCCCGCACCTCGTCC TCCAACCCCTGCACCTACGATTGCCAGTC AGCCTCTTTCCTGCGGCCTGAGGCCAGC AGACCAGCTGCCGGCGGTGCCGTCCATAC AAGAGGACTGGACTTCGCGTCCGATAAAC CTACTACCACTCCAGCCCCAAGGCCCCCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		RPPTPAPTIASQPLSLRP EACRPAAGGAVHTRGL DFACD		ACCCAGCACCGACTATCGCATCACAGCC TTTGTCACTGCGTCCTGAAGCCAGCCGGC CAGCTGCAGGGGGGGCCGTCACACAAG GGGACTCGACTTTGCGAGTGATAAGCCCA CCACCACCCCTGCCCCCTAGACCTCCAACC CCAGCCCCTACAATCGCCAGCCAGCCCCT GAGCCTGAGGCCCCGAAGCCTGTAGACCTG CCGCTGGCGGAGCCGTGCACACCAGAGG CCTGGATTTCGCCTGCGAC
CD8alpha 4x	60	TTPAPRPPTPAPTIASQP LSLRPEASRPAAGGAV HTRGLDFASDKPTTTPA PRPPTPAPTIASQPLSLR PEASRPAAGGAVHTRG LDFASDKPTTTPAPRPP TPAPTIASQPLSLRPEAS RPAAGGAVHTRGLDFA SDKPTTTPAPRPPTPAPT IASQPLSLRPEACRPAA GGAVHTRGLDFACD	91	AAGCCTACCACCACCCCCGCACCTCGTCC TCCAACCCCTGCACCTACGATTGCCAGTC AGCCTCTTTCCTGCGGCCTGAGGCCAGC AGACCAGCTGCCGGCGGTGCCGTCCATAC AAGAGGACTGGACTTCGCGTCCGATAAAC CTACTACCACTCCAGCCCCAAGGCCCCCA ACCCAGCACCGACTATCGCATCACAGCC TTTGTCACTGCGTCCTGAAGCCAGCCGGC CAGCTGCAGGGGGGGCCGTCACACAAG GGGACTCGACTTTGCGAGTGATAAACCTA CTACAACCTCCTGCCCCCGGCCTCCTACA CCAGCTCCTACTATCGCCTCCCAGCCACT CAGTCTCAGACCCGAGGCTTCTAGGCCAG CGGCCGGAGGCGCGGTCCACACCCGCGG GCTGGACTTTGCATCCGATAAGCCCACCA CCACCCCTGCCCCCTAGACCTCCAACCCCA GCCCCCTACAATCGCCAGCCAGCCCCTGAG CCTGAGGCCCCGAAGCCTGTAGACCTGCCG CTGGCGGAGCCGTGCACACCAGAGGCCTG GATTTCGCCTGCGAC
CD8alpha TM	61	IYIWAPLAGTCGVLLLS LVITLYCNHRN	92	ATCTACATCTGGGCCCTCTGGCCGGCAC CTGTGGCGTGCTGCTGCTGAGCCTGGTCA TCACCCTGTACTGCAACCACCGGAAT
CD28 TM	62	FWVLVVVGGVLACYSL LVTVAFIIFWV	93	TTTTGGGTGCTGGTGGTGGTTGGTGGAGT CCTGGCTTGCTATAGCTTGCTAGTAACAG TGGCCTTTATTATTTTCTGGGTG
4-1BB signaling domain	63	KRGRKKLLYIFKQPFM RPVQTTQEEDGCSCRF EEEEGGCEL	94	AAGAGAGGCCGGAAGAACTGCTGTACA TCTTCAAGCAGCCCTTCATGCGGCCCGTG CAGACCACCCAGGAAGAGGACGGCTGCA GCTGCCGGTTCCCCGAGGAAGAGGAAGG CGGCTGCGAACTG
CD28 signaling domain	64	RSKRSRGGHSDYMN TPRRPGPTRKHYQPYAP PRDFAAYRS	95	AGGAGCAAGCGGAGCAGAGGCGGCCACA GCGACTACATGAACATGACCCCCCGGAGG CCTGGCCCCACCCGGAAGCACTACCAGCC CTACGCCCTCCCAGGGACTTCGCCGCCT ACCGGAGC
DNAX- activation protein 10 (DAP 10) Signaling Domain	65	LCARPRRSPAQEDGKV YINMPGRG	96	CTGTGCGCACGCCCACGCCGAGCCCCGC CCAAGAAGATGGCAAAGTCTACATCAAC ATGCCAGGCAGGGGC
DNAX- activation	66	YFLGRLVPRGRGAAEA ATRKQRITETESPYQEL QGQRSDVYSDLNTQRP YYK	97	TACTTCCTGGGCCGGCTGGTCCCTCGGGG GCGAGGGGCTGCGGAGGCAGCGACCCGG AAACAGCGTATCACTGAGACCGAGTCGCC TTATCAGGAGCTCCAGGGTCAGAGGTCGG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
protein 12 (DAP12) Signaling Domain				ATGTCTACAGCGACCTCAACACACAGAGG CCGTATTACAAA
CD3 ζ signaling domain	67	RVKFSRSADAPAYQQG QNQLYNELNLGRREEY DVLDKRRGRDPEMGG KPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGE RRRGKGHDGLYQGLST ATKDTYDALHMQALPP R	98	CGGGTGAAGTTCAGCCGGAGCGCCGACG CCCCTGCCTACCAGCAGGGCCAGAACCAG CTGTACAACGAGCTGAACCTGGGCGGAG GGAGGAGTACGACGTGCTGGACAAGCGG AGAGGCCGGGACCCTGAGATGGGCGGCA AGCCCCGGAGAAAGAACCCTCAGGAGGG CCTGTATAACGAACTGCAGAAAGACAAG ATGGCCGAGGCCTACAGCGAGATCGGCAT GAAGGGCGAGCGGCGGAGGGGCAAGGGC CACGACGGCCTGTACCAGGGCCTGAGCAC CGCCACCAAGGATACCTACGACGCCCTGC ACATGCAGGCCCTGCCCCCAGA
HER1t	68	RKVCNGIGIGEFKDSL SI NATNIKHFKNCTSI SGD LHILPVAFRGDSFT HTP PLDPQELDILKTVKE IT GFLLIQAWPENRTDL H AFENLEIIRGRTKQH GQ FSLAVVSLNITSLGL RSL KEISDGDVIISGNKN LC YANTINWKKLFGTSG Q KTKIISNRGENSCKA TG QVCHALCSPEGCWGP E PRDCVSCRNVSRGRE C VDKCNLLEGEPREFV E NSECIQCHPECLPQA MN ITCTGRGPDNCIQCA HY IDGPHCVKTCPAGVM G ENNTLVWKYADAGHV CHLCHPNCTYGCTGP G LEGCP TNGPKIPSIAT G MVGALLLLLVVALGIG LFM	99	CGCAAAGTGTGTAACGGAATAGGTATTGG TGAATTTAAAGACTCACTCTCCATAAATG CTACGAATATTAACACTTCAAAA ACTGC ACCTCCATCAGTGGCGATCTCCACATCCT GCCGGTGGCATT TAGGGGTGACTCCTTCA CACATACTCCTCCTCTGGATCCACAGGAA CTGGATATTCTGAAAACCGTAAAGGAAAT CACAGGGTTTTTGTCTGATTCAGGCTTGGC CTGAAAACAGGACGGACCTCCATGCCTTT GAGAACCTAGAAATCATAACGCGGCAGGA CCAAGCAACATGGTCAGTTTTCTCTTGCA GTCGTCAGCCTGAACATAACATCCTTGGG ATTACGCTCCCTCAAGGAGATAAGTGATG GAGATGTGATAATTT CAGGAAACAAAAAT TTGTGCTATGCAAATACAATAAACTGGAA AAA ACTGTTTGGGACCTCCGGTCAGAAAA CCAAAATTATAAGCAACAGAGGTGAAAA CAGCTGCAAGGCCACAGGCCAGGTCTGCC ATGCCTTGTGCTCCCCCGAGGGCTGCTGG GGCCCGGAGCCCAGGGACTGCGTCTCTTG CCGGAATGTCAGCCGAGGCAGGGAATGC GTGGACAAGTGCAACCTTCTGGAGGGTGA GCCAAGGGAGTTTGTGGAGAACTCTGAGT GCATACAGTGCCACCCAGAGTGCCTGCCT CAGGCCATGAACATCACCTGCACAGGACG GGGACCAGACAACTGTATCCAGTGTGCCC ACTACATTGACGGCCCCCACTGCGTCAAG ACCTGCCCCGGCAGGAGTCATGGGAGAAA ACAACACCCTGGTCTGGAAGTACGCAGAC GCCGGCCATGTGTGCCACCTGTGCCATCC AAACTGCACCTACGGATGCACTGGGCCAG GTCTTGAAGGCTGTCCAACGAATGGGCCT AAGATCCCGTCCATCGCCACTGGGATGGT GGGGGCCCTCCTCTTGCTGCTGGTGGTGG CCCTGGGGATCGGCCTCTTCATG
HER1t- 1	69	RKVCNGIGIGEFKDSL SI NATNIKHFKNCTSI SGD LHILPVAFRGDSFT HTP PLDPQELDILKTVKE IT GFLLIQAWPENRTDL H	100	CGCAAAGTGTGTAACGGAATAGGTATTGG TGAATTTAAAGACTCACTCTCCATAAATG CTACGAATATTAACACTTCAAAA ACTGC ACCTCCATCAGTGGCGATCTCCACATCCT GCCGGTGGCATT TAGGGGTGACTCCTTCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		AFENLEIIRGRTKQHGQ FSLAVVSLNITSLGLRSL KEISDGDVIISGNKNLC YANTINWKKLFGTSGQ KTKIISNRGENSCKATG QVCHALCSPEGCWGPE PRDCVSGGGGSGGGSG GGSGGGGGSFWVLVV VGGVLACYSLLVTVAFI IFWVRSKRS		CACATACTCCTCCTCTGGATCCACAGGAA CTGGATATTCTGAAAACCGTAAAGGAAAT CACAGGGTTTTTGTCTGATTCAGGCTTGGC CTGAAAACAGGACGGACCTCCATGCCTTT GAGAACCTAGAAATCATACGCGGCAGGA CCAAGCAACATGGTCAGTTTTTCTCTTGCA GTCGTCAGCCTGAACATAACATCCTTGGG ATTACGCTCCCTCAAGGAGATAAGTGATG GAGATGTGATAATTTTCAGGAAACAAAAAT TTGTGCTATGCAAATACAATAAACTGGAA AAAACGTGTTTGGGACCTCCGGTCAGAAAA CCAAAATTATAAGCAACAGAGGTGAAAA CAGCTGCAAGGCCACAGGCCAGGTCTGCC ATGCCTTGTGCTCCCCCGAGGGCTGCTGG GGCCCGGAGCCCAGGGACTGCGTCTCTGG TGGCGGTGGCTCGGGCGGTGGTGGGTCTGG GTGGCGGCGGATCTGGTGGCGGTGGCTCG TTTTGGGTGCTGGTGGTGGTTGGTGGAGT CCTGGCTTGCTATAGCTTGCTAGTAACAG TGGCCTTTATTATTTTCTGGGTGAGGAGTA AGAGGAGC
FL CD20	70	MTTPRNSVNGTFPAEP MKGPIAMQSGPKPLFR RMSSLVGPTQSFFMRES KTLGAVQIMNGLFHIAL GGLLMIPAGIYAPICVT VWYPLWGGIMYIISGSL LAATEKNSRKCLVKGK MIMNSLSLFAAISGMIL SIMDILNIKISHFLKMES LNFIRAHTPYINIYNCEP ANPSEKNSPSTQYCYSI QSLFLGILSVMLIFAFFQ ELVIAGIVENEWKRTCS RPKSNIVLLSAEEKKEQ TIEIKEEVVGLTETSSQP KNEEDIEIPIQEEEEET ETNFPEPPQDQESSPIEN DSSP	101	ATGACAACACCCAGAAATTCAGTAAATGG GACTTTCCCGGCAGAGCCAATGAAAGGCC CTATTGCTATGCAATCTGGTCCAAAACCA CTCTTCAGGAGGATGTCTTCACTGGTGGG CCCCACGCAAAGCTTCTTCATGAGGGAAT CTAAGACTTTGGGGGCTGTCCAGATTATG AATGGGCTCTTCCACATTGCCCTGGGGGG TCTTCTGATGATCCCAGCAGGGATCTATG CACCCATCTGTGTGACTGTGTGGTACCCT CTCTGGGGAGGCATTATGTATATTATTTCC GGATCACTCCTGGCAGCAACGGAGAAAA ACTCCAGGAAGTGTTTGGTCAAAGGAAAA ATGATAATGAATTCATTGAGCCTCTTTGCT GCCATTTCTGGAATGATTCTTTCAATCATG GACATACTTAATATTAATAATTTCCCATT TTAAAAATGGAGAGTCTGAATTTTATTAG AGCTCACACACCATATATTAACATATACA ACTGTGAACCAGCTAATCCCTCTGAGAAA AACTCCCCATCTACCCAATACTGTTACAG CATACAATCTCTGTTCTTGGGCATTTTGT AGTGATGCTGATCTTTGCCTTCTTCCAGGA ACTTGTAATAGCTGGCATCGTTGAGAATG AATGGAAAAGAACGTGCTCCAGACCCAA ATCTAACATAGTTCTCCTGTCAGCAGAAG AAAAAAAAGAACAGACTATTGAAATAAA AGAAGAAGTGGTTGGGCTAACTGAAACA TCTTCCCAACCAAAGAATGAAGAAGACAT TGAAATTATTCCAATCCAAGAAGAGGAAG AAGAAGAAACAGAGACGAACTTTCCAGA ACCTCCCCAAGATCAGGAATCCTCACCAA TAGAAAATGACAGCTCTCCT
CD20t-1	71	MTTPRNSVNGTFPAEP MKGPIAMQSGPKPLFR RMSSLVGPTQSFFMRES KTLGAVQIMNGLFHIAL GGLLMIPAGIYAPICVT VWYPLWGGIMYIISGSL	102	ATGACCACACCACGGAACCTCTGTGAATGG CACCTTCCCAGCAGAGCCAATGAAGGGAC CAATCGCAATGCAGAGCGGACCCAAGCCT CTGTTTCGGAGAATGAGCTCCCTGGTGGG CCCAACCCAGTCCTTCTTTATGAGAGAGT CTAAGACACTGGGCGCCGTGCAGATCATG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		LAATEKNSRKCLVKGK MIMNSLSLFAAISGMIL SIMDILNIKISHFLKMES LNFIRAHTPYINIYNCEP ANPSEKNSPSTQYCYSI QSLFLGILSVMLIFAFFQ ELVIAGIVENEWKRTCS RPKSNIVLLSAEEKKEQ TIEIKEEVVGLTETSSQP KNEEDIE		AACGGACTGTTCCACATCGCCCTGGGAGG ACTGCTGATGATCCCAGCCGGCATCTACG CCCCTATCTGCGTGACCGTGTGGTACCCT CTGTGGGGCGGCATCATGTATATCATCTC CGGCTCTCTGCTGGCCGCCACAGAGAAGA ACAGCAGGAAGTGTCTGGTGAAGGGCAA GATGATCATGAATAGCCTGTCCCTGTTTG CCGCCATCTCTGGCATGATCCTGAGCATC ATGGACATCCTGAACATCAAGATCAGCCA CTTCCTGAAGATGGAGAGCCTGAACTTCA TCAGAGCCCACACCCCTTACATCAACATC TATAATTGCGAGCCTGCCAACCCATCCGA GAAGAATTCTCCAAGCACACAGTACTGTT ATTCCATCCAGTCTCTGTTCTTGGGCATCC TGTCTGTGATGCTGATCTTTGCCTTCTTTC AGGAGCTGGTCATCGCCGGCATCGTGGAG AACGAGTGGAAGAGGACCTGCAGCCGCC CCAAGTCCAATATCGTGCTGCTGTCCGCC GAGGAGAAGAAGGAGCAGACAATCGAGA TCAAGGAGGAGGTGGTGGGCCTGACCGA GACATCTAGCCAGCCTAAGAATGAGGAG GATATCGAG
mbIL15	103	MDWTWILFLVAAATR VHSNWVNVISDLKKIE DLIQSMHIDATLYTESD VHPSCKVTAMKCFLE LQVISLESGDASIHDTV ENLIILANNSLSSNGNV TESGCKECELEEKNIK EFLQSFVHIVQMFINTSS GGSGGGGSGGGGSGG GGSGGGSLQITCPPPMS VEHADIWVKSYSLYSR ERYICNSGFKRKAGTSS LTECVLNKATNVAHWT TPSLKCIRDPALVHQR APPSTVTTAGVTPQPES LSPSGKEPAASSPSSNN TAATTAAIVPGSQLMPS KSPSTGTTEISSHESHG TPSQTTAKNWELTASA SHQPPGVYPQGHSDTT VAISTSTVLLCGLSAVS LLACYLKSRQTPPLASV EMEAMEALPVTWGTSS RDEDLENCSHHL	106	ATGGATTGGACCTGGATTCTGTTTCTGGT GGCCGCTGCCACAAGAGTGCACAGCAACT GGGTGAATGTGATCAGCGACCTGAAGAA GATCGAGGATCTGATCCAGAGCATGCACA TTGATGCCACCCTGTACACAGAATCTGAT GTGCACCCTAGCTGTAAAGTGACCGCCAT GAAGTGTTTTCTGCTGGAGCTGCAGGTGA TTTCTCTGGAAAGCGGAGATGCCTCTATC CACGACACAGTGGAGAATCTGATCATCCT GGCCAACAATAGCCTGAGCAGCAATGGC AATGTGACAGAGTCTGGCTGTAAGGAGTG TGAGGAGCTGGAGGAGAAGAACATCAAG GAGTTTCTGCAGAGCTTTGTGCACATCGT GCAGATGTTTCATCAATAACAAGCTCTGGCG GAGGATCTGGAGGAGGCGGATCTGGAGG AGGAGGCAGTGGAGGCGGAGGATCTGGC GGAGGATCTCTGCAGATTACATGCCCTCC TCCAATGTCTGTGGAGCACGCCGATATTT GGGTGAAGTCCTACAGCCTGTACAGCAGA GAGAGATACATCTGCAACAGCGGCTTTAA GAGAAAGGCCGGCACCTCTTCTCTGACAG AGTGCGTGCTGAATAAGGCCACAAATGTG GCCCACTGGACAACACCTAGCCTGAAGTG CATTAGAGATCCTGCCCTGGTCCACCAGA GGCCTGCCCCCTCCATCTACAGTGACAACA GCCGGAGTGACACCTCAGCCTGAATCTCT GAGCCCTTCTGGAAAAGAACCTGCCGCCA GCTCTCCTAGCTCTAATAATACCGCCGCC ACAACAGCCGCCATTGTGCCTGGATCTCA GCTGATGCCTAGCAAGTCTCCTAGCACAG GCACAACAGAGATCAGCAGCCACGAATC TTCTCACGGAACACCTTCTCAGACCACCG CCAAGAATTGGGAGCTGACAGCCTCTGCC TCTCACCAGCCTCCAGGAGTGTATCCTCA GGGCCACTCTGATACAACAGTGGCCATCA GCACATCTACAGTGCTGCTGTGTGGACTG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				TCTGCCCGTGTCTCTGCTGGCCTGTTACCTG AAGTCTAGACAGACACCTCCTCTGGCCTC TGTGGAGATGGAGGCCATGGAAGCCCTGC CTGTGACATGGGGAACAAGCAGCAGAGA TGAGGACCTGGAGAATTGTTCTCACCACC TG
IL-15	104	NWVNVISDLKKIEDLIQ SMHIDATLYTESDVHPS CKVTAMKCFLLLELQVIS LESGDASIHDVTENLIIL ANNSLSSNGNVTESGC KECEELEEKNIKEFLQS FVHIVQMFINTS	107	AACTGGGTGAATGTGATCAGCGACCTGAA GAAGATCGAGGATCTGATCCAGAGCATGC ACATTGATGCCACCCTGTACACAGAATCT GATGTGCACCCTAGCTGTAAAGTGACCGC CATGAAGTGTTTTCTGCTGGAGCTGCAGG TGATTTCTCTGGAAAGCGGAGATGCCTCT ATCCACGACACAGTGGAGAATCTGATCAT CCTGGCCAACAATAGCCTGAGCAGCAATG GCAATGTGACAGAGTCTGGCTGTAAGGAG TGTGAGGAGCTGGAGGAGAAGAACATCA AGGAGTTTCTGCAGAGCTTTGTGCACATC GTGCAGATGTTTCATCAATAACAAGC
IL-15R α	105	ITCPPPMSVEHADIWVK SYSLYSRERYICNSGFK RKAGTSSLTECVLNKA TNVAHWTTPSLKCIRDP ALVHQRPAAPPSTVTTA GVTPQPESLSPSGKEPA ASSPSSNNTAATTAAIV PGSQLMPSKSPSTGTTEI SSHESSHGTPSQTTAKN WELTASASHQPPGVYP QGHSDTTVAISTSTVLL CGLSAVSLLACYLKSR QTPPLASVEMEAMEAL PVTWGTSSRDEDLENC SHHL	108	ATTACATGCCCTCCTCCAATGTCTGTGGA GCACGCCGATATTTGGGTGAAGTCCTACA GCCTGTACAGCAGAGAGAGATACATCTGC AACAGCGGCTTTAAGAGAAAGGCCGGCA CCTCTTCTCTGACAGAGTGCGTGCTGAAT AAGGCCACAAATGTGGCCCACTGGACAA CACCTAGCCTGAAGTGCATTAGAGATCCT GCCCTGGTCCACCAGAGGCCTGCCCTCC ATCTACAGTGACAACAGCCGGAGTGACAC CTCAGCCTGAATCTCTGAGCCCTTCTGGA AAAGAACCTGCCGCCAGCTCTCCTAGCTC TAATAATACCGCCGCCACAACAGCCGCCA TTGTGCCTGGATCTCAGCTGATGCCTAGC AAGTCTCCTAGCACAGGCACAACAGAGAT CAGCAGCCACGAATCTTCTCACGGAACAC CTTCTCAGACCACCGCCAAGAATTGGGAG CTGACAGCCTCTGCCTCTCACCAGCCTCC AGGAGTGTATCCTCAGGGCCACTCTGATA CAACAGTGGCCATCAGCACATCTACAGTG CTGCTGTGTGGACTGTCTGCCGTGTCTCTG CTGGCCTGTTACCTGAAGTCTAGACAGAC ACCTCCTCTGGCCTCTGTGGAGATGGAGG CCATGGAAGCCCTGCCTGTGACATGGGGA ACAAGCAGCAGAGATGAGGACCTGGAGA ATTGTTCTCACCACCTG
HUMAN Immunogl obulin heavy constant gamma 4 (IgG4 CH)	146	ASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFP EPVTVSWNSGALTSGV HTFPAVLQSS GLYSLSSVVTVPSSSLG TKTYTCNVDHKPSNTK VDKRVESKYGPPCPSCP APEFLGGPSV FLFPPKPKDTLMISRTPE VTCVVVDVSQEDPEVQ FNWYVDGVEVHNAKT KPREEQFNSTY RVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVY		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		TLPPSQEEMTK NQVSLTCLVKGFYPSDI AVEWESNGQPENNYKT TPPVLDSDGSFFLYSRL TVDKSRWQEG NVFSCSVMHEALHNHY TQKSLSLSLGK		
IgG4 CH (S108P, L325P)	147	ASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFP EPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSS VVTVPSSSLGTKTYTCN VDHKPSNTKVDKRVES KYGPPCPPCPAPEFLGG PSVFLFPPKPKDTLMISR TPEVTCVVVDVSQEDP EVQFNWYVDGVEVHN AKTKPREEQFNSTYRV VSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKT ISKAKGQPREPQVYTL PSQEEMTKNQVSLTCL VKGFYPSDIAVEWESN GQPENNYKTTPPVLD SDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEAL HNHYTQKSLSLSPGK	117	GCTAGCACCAAGGGCCCATCGGTCTTCCC CCTGGCGCCCTGCTCCAGGAGCACCTCCG AGAGCACAGCCGCCCTGGGCTGCCTGGTC AAGGACTACTTCCCCGAACCGGTGACGGT GTCGTGGAACCTCAGGCGCCCTGACCAGCG GCGTGACACACCTTCCCGGCTGTCCTACAG TCCTCAGGACTCTACTCCCTCAGCAGCGT GGTGACCGTGCCCTCCAGCAGCTTGGGCA CGAAGACCTACACCTGCAACGTAGATCAC AAGCCCAGCAACACCAAGGTGGACAAGA GAGTTGAGTCCAAATATGGTCCCCCATGC CCACCATGCCCAGCACCTGAGTTCCTGGG GGGACCATCAGTCTTCCTGTTCCCCCCAA AACCCAAGGACACTCTCATGATCTCCCGG ACCCCTGAGGTCACGTGCGTGGTGGTGG CGTGAGCCAGGAAGACCCCGAGGTCCAG TTCAACTGGTACGTGGATGGCGTGGAGGT GCATAATGCCAAGACAAAGCCGCGGGAG GAGCAGTTCAACAGCACGTACCGTGTGGT CAGCGTCCTCACCGTCCTGCACCAGGACT GGCTGAACGGCAAGGAGTACAAGTGCAA GGTCTCCAACAAAGGCCTCCCGTCCTCCA TCGAGAAAACCATCTCCAAAGCCAAAGG GCAGCCCCGAGAGCCACAGGTGTACACCC TGCCCCCATCCCAGGAGGAGATGACCAAG AACCAGGTCAGCCTGACCTGCCTGGTCAA AGGCTTCTACCCAGCGACATCGCCGTGG AGTGGGAGAGCAATGGGCAGCCGGAGAA CAACTACAAGACCACGCCTCCCGTGCTGG ACTCCGACGGCTCCTTCTTCTCTACAGCA GGCTCACCGTGGACAAGAGCAGGTGGCA GGAGGGGAATGTCTTCTCATGCTCCGTGA TGCATGAGGCTCTGCACAACCACTACACA CAGAAGAGCCTCTCCCTGTCTCCGGGTAA A
IgG4 CH (S108P)	291	ASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFP EPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSS VVTVPSSSLGTKTYTCN VDHKPSNTKVDKRVES KYGPPCPPCPAPEFLGG PSVFLFPPKPKDTLMISR TPEVTCVVVDVSQEDP EVQFNWYVDGVEVHN AKTKPREEQFNSTYRV VSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKT ISKAKGQPREPQVYTL PSQEEMTKNQVSLTCL VKGFYPSDIAVEWESN		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		GQPENNYKTTPPVLDS DGSFFLYSRLTVDKSR WQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK		
IgG4 CH (L325P)	292	ASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFP EPVTVSWNSGALTSGV HTFPAVLQSS GLYSLSSVVTVPSSSLG TKTYTCNVDPHKPSNTK VDKRVESKYGPCCPSCP APEFLGGPSV FLFPPKPKDTLMISRTPE VTCVVVDVSQEDPEVQ FNWYVDGVEVHNAKT KPREEQFNSTY RVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVY TLPPSQEEMTK NQVSLTCLVKGFYPSDI AVEWESNGQPENNYKT TPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHEALHNHY TQKSLSLSPGK		
Anti-PD1 (nVL1)			118	gagatagttatgactcaaagccccgctacattatccctgtctccgggt gaacgggccaccctgtcatgccgggcttcacagtcagtgtcaagct atctggcatggtatcagcagaagcctggacaggccccaaggctact gattatgacgccagcaaccgcgctacaggtattcctgctaggttctc agggtcaggctctggaaccgactttactctgactatctcctctctgaa cccgaggatttcgcggtgtactactgtcagcagtagataataactggcca cgcacattcggccagggcactaaagtcgaaattaag
Anti-PD1 (nVL2)			119	gagatcgtactgactcagtcctccagccacattgtccctgtccccagg ggagcgcgccaccctgagctgtagagcttcacagtcctcagttctt acctcgcgtggtatcagcaaaaacctggacaagctccgaggttgctt atctatgacgctccaaccgcgccactggcataccagcaaggttca gcggatctgggtccggcacagattttaccctcactatttctagcctga gccggaagatttcgctgtttactactgccagcagcgateccaactggc ccaagacattcggccagggaactaaagtggaaatcaaa
Anti-PD1 (nVL3)	148	EIVLTQSPATLSLSPGERAT LSCRASQSVSSYLAWYQQKP GQAPRLLIYDASNRANGIPA RFSGSGSGTDFTLTISSELP EDFAVYYCQQSSNWPRTFGQ GTKVEIKR	120	gaaattgtgtgacacagtcctccagccaccctgtcttgtctccagg gaaagagccaccctctcctgcagggccagtcagagtgttagtagtta cttagcctggtaccaacagaaacctggccagggtccaggtcctc atctatgatgcaccaacagggccaacggcatccagccaggttca gtggcagtggtctgtggacagacttcactctaccatcagcagccta gagcctgaagatttcgagtttattactgtcagcagagtagcaactgg cctcggacgttcggccaagggaaccaaggtggaaatcaaaaga
Anti-PD1 (pVL1)			121	cggaacgtgctgacccagtccccccttagcctcccc gtcacgcccggagagcccgaagtatcagctgccgc agttcacaaagtctgagttcttctggatacacctat ttggactggtattttgcagaagccagggaatcccca cagctcctgatatacctcgaagctggagagatagc ggagtacctgatcgtttttctggtagcggatctggt acggatttcactctgaagatttctagggtggaggcg gaggacgtgggagtgactactgtatgcaagccgag cagactcccggcccaggtaacacgttcggacagggg accaaactggagattaag

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
Anti-PD1 (pVL2)			122	gatgtggtaatgacccagtcacctctttcactgcct gtcactcccggagagccagcttcaatctcctgccgt agctctcaatcattgttgcacaccaatggatacaac tacctccactggtatctccagaagcccggacaaagc ccgcagctgctgatctacctgggcagctggcaggac tccgggggtgcccgaaccgatttagcggcagtgaggc ggcacggactttacactgaagatcagccgagtagag gcggaggacgtgggcgtttactactgtatgcaggca gagcagacccccagaaccttcggccagggcacccgg ctggagggtgaaa
Anti-PD1 (nVH1)			123	caggtgcagttggttgaaagcggaggaggcgtgggt caaccggtagaagcctacggctgtcatgtgcggcc tccggcttcacatttcgatcttacggaatgcactgg gtcaggcaggcaccggcaagggtctggagtgggtc gccataattttctatgacggcagcaacaagtattac gccgacagtgttaaggggcggtttaccatcagcaga gacaactctaaaaacactctttatctgcaaataaac tctctgcgggcagaggataaccgctgtttactattgc gccagagatgacgactactgggggcagggtgccttg gtgactgtgagcagc
Anti-PD1 (nVH2)			124	gaggtccagttagtccaaagcggcggaggcgtagt caacctggcagaagcctgcgggttatcgtgcgccga agcggcttcaccttttagctcttatggtatgcactgg gtcagacaggccccctgggaaggccctggagtgggtg gccgtgatctggtatgacgggagcaacaagtattac gcggattccgtcaagggacgggttcaccatatccgc gataacagcaagaataactctttacttacagatgaac agcctgaggggccgaggacaccgcagtatatattgc gctggcgaaggctttgactattgggggtcagggcact ctggtgactgtgagcagc
Anti-PD1 (nVH3)	149	QVQLVESGGGVVQPGRSLRL DCKASGITFSNSGMHWVRQA PGKGLEWVAVIWDGSKRYY ADSVKGRFTISRDN SKNTLF LQMNSLRAEDTAVYYCATNN DYWGQGT LVT VSS	125	Caggtgcagctggtggagtctgggggaggcgtgggtc cagcctgggaggtccctgagactcgactgtaaagcg tctggaatcaccttcagtaactctggcatgcactgg gtccgccaggctccaggcaaggggctggagtgggtg gcagttattttggtatgatggaagtaaaagatactat gcagactccgtgaagggccgattcaccatctccaga gacaattccaagaacacgctgtttctgcaaataaac agcctgagagccgaggacacggctgtgtattactgt gcgacaaacaacgactactggggccagggaaccctg gtcaccgtctcctca
Anti-PD1 (nVH4)	150	QVQLVESGGGVVQPGRSLRL DCKASGITFSNSGMHWVRQA PGKGLEWVAVIWDGSKRYY ADSVKGRFTISRDN SKNTLF LQMNSLRAEDTAVYYCARND DYWGQGT LVT VSS	126	caggtgcagctggtggagtctgggggaggcgtgggtc cagcctgggaggtccctgagactcgactgtaaagcg tctggaatcaccttcagtaactctggcatgcactgg gtccgccaggctccaggcaaggggctggagtgggtg gcagttattttggtatgatggaagtaaaagatactat gcagactccgtgaagggccgattcaccatctccaga gacaattccaagaacacgctgtttctgcaaataaac agcctgagagccgaggacacggctgtgtattactgt gcgagaaacgacgactactggggccagggaaccctg gtcaccgtctcctca
Anti-PD1 (pVH1)	151	QVQLVQSGAEVKKPGASVKV SCKASGYTFTGYMHWRQA PGQGLEWMGAINPNSGGTNY AQKFQGRVTMTRDTSISTAY	127	Caggtacagctggtgcagagcggcgagaggtgaag aagccaggcgcttctgtaaagggtatcctgcaaggca tccgggtatactttcaccggctattacatgcactgg gttcgtcaggcaccggccagggactagaatggatg

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		MELSLRSDDTAVYYCARRP DRANWHFDYWGQGLTVTVSS		ggggccatcaaccctaatagtggcggtactaactac gcacaaaagtttcagggcgagtgaccatgactcgg gatacctccatctccacggcatacatggagctgagt cgcttgcggtcagatgacactgcggtgtactactgc gctcgcaggcccgaccgagctaattggcactttgac tactggggacagggtacactggtgaccgtgtcatca
Anti-PD1 (pVH2)	152	QVQLVQSGAEVKKPGASVKV SCKASGYTFTGYMHWRQA PGQGLEWMGAINPNSGGTNY AQKLQGRVTMTTDTSTSTAY MELSLRSDDTAVYYCARHG LKGDGYFDYWGQGLTVTVSS	128	Caggtgcagctggtccagagcggcgcggaagtgaaa aagcccgcgcttccgtgaaggtttcttgcaaagcc tctggatacacattcactggctattatatgcactgg gtcagacaggcccccgccagggttgagtgatg ggtgcaatcaaccccaattctggtgggaccaattac gcacagaaactccagggccgagtgacaatgaccacc gacacttctaccagcactgcctacatggagctgcgg tctctgcgatcagacgacaccgctgtgtactattgt gcaagacacgggctgaaggcgacggctattttgac tactggggacagggcacgctggttaccgtgagttcc
Anti-PD1 (pVH3)	153	QVQLVQSGVEVKKPGASVKV SCKASGYTFTNYMYWVRQA PGQGLEWMGGINPSNGGTNF NEKFKNRVTLTDSSTTTAY MELKSLQFDDTAVYYCARRD YRFYMGFDYWGQGT TVTVSS	129	caggtccagctcgtgcaaagcggagtggaagtgaaa aagcctggcgcttccgtcaaggctcagctgtaaggcc agcggatacacattcacaaactattacatgtactgg gtgaggcaggctcccggacagggactggaatggatg ggcggaatcaatccctccaacggaggcacaaacttt aacgaaaagtttaagaatagagtcaccctcaccaca gactccagcacaccacagcctatatggaactgaaa agcctccagtttgacgataccgctgtgtattactgt gccaggagagattacaggttctacatgggattcgat tactggggccaaggcacaaccgtcaccgtcagctcc
Anti-PD1 (pVH4)	154	QVQLVQSGVEVKKPGASVKV SCKASGYTFTNYMYWVRQA PGQGLEWMGGINPSNGGTNF NEKFKNRVTLTDSSTTTAY MELKSLQFDDTAVYYCARRD YRFNMGFDYWGQGT TVTVSS	130	caggtccagctcgtgcaaagcggagtggaagtgaaa aagcctggcgcttccgtcaaggctcagctgtaaggcc agcggatacacattcacaaactattacatgtactgg gtgaggcaggctcccggacagggactggaatggatg ggcggaatcaatccctccaacggaggcacaaacttt aacgaaaagtttaagaatagagtcaccctcaccaca gactccagcacaccacagcctatatggaactgaaa agcctccagtttgacgataccgctgtgtattactgt gccaggagagattacaggttcaacatgggattcgat tactggggccaaggcacaaccgtcaccgtcagctcc
Anti-PD1 (pVH5)	155	QVQLVQSGVEVKKPGASVKV SCKASGYTFTNYMYWVRQA PGQGLEWMGGIQPSNGGTNF NEKFKNRVTLTDSSTTTAY MELKSLQFDDTAVYYCARRD YRFYMGFDYWGQGT TVTVSS	131	Caggtccagctcgtgcaaagcggagtggaagtgaaa aagcctggcgcttccgtcaaggctcagctgtaaggcc agcggatacacattcacaaactattacatgtactgg gtgaggcaggctcccggacagggactggaatggatg ggcggaatccagccctccaacggaggcacaaacttt aacgaaaagtttaagaatagagtcaccctcaccaca gactccagcacaccacagcctatatggaactgaaa agcctccagtttgacgataccgctgtgtattactgt gccaggagagattacaggttctacatgggattcgat tactggggccaaggcacaaccgtcaccgtcagctcc
Anti-PD1 (pVH6)	156	QVQLVQSGVEVKKPGASVKV SCKASGYTFTNYMYWVRQA PGQGLEWMGGIDPSNGGTNF NEKFKNRVTLTDSSTTTAY MELKSLQFDDTAVYYCARYD YRFDMGFDYWGQGT TVTVSS	132	Caggtccagctcgtgcaaagcggagtggaagtgaaa aagcctggcgcttccgtcaaggctcagctgtaaggcc agcggatacacattcacaaactattacatgtactgg gtgaggcaggctcccggacagggactggaatggatg ggcggaatcgacccctccaacggaggcacaaacttt aacgaaaagtttaagaatagagtcaccctcaccaca

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				gactccagcacaaccacagcctatatggaactgaaa agcctccagtttgacgataccgctgtgtattactgt gccagggtacgattacaggttcgatatgggattcgat tactggggccaaggcacaaccgtcaccgtcagctcc
Anti-PD1 (nVH7)	157	QVQLVESGGGVVQPGRSLRL DCKASGFTFSNSGMHWVRQA PGKGLEWVAVIWYDGSKRYY ADSVKGRFTISRDN SKNTLF LQMNSLRAEDTAVYYCATNN DYWGQGT LVT VSS	133	cagggtgcagctgggtggagtctgggggaggcgtggtc cagcctgggagggtccctgagactcgactgtaaagcg tctggattcaccttcagtaactctggcatgcactgg gtccgccagggtccaggcaaggggctggagtgggtg gcagttatttggtatgatggaagtaaaagatactat gcagactccgtgaagggccgattcaccatctccaga gacaattccaagaacacgctgtttctgcaaataaac agcctgagagccgaggacacggctgtgtattactgt gcgacaaacaacgactactggggccagggaaccctg gtcaccgtctcctca
Anti-PD1 (nVH8)	158	EVQLVQSGGGVVQPGRSLRL SCAASGFTFSYGMHWVRQA PGKGLEWVAVIWYDGSNKYY ADSVKGRFTISRDN SKNTLY LQMNSLRAEDTAVYYCATNN DYWGQGT LVT VSS	134	gagggtccagtttagtccaaagcggcggaggcgttagtg caacctggcagaagcctgcggttatcgtgcgccgca agcggcttcaccttttagctcttatggtatgcactgg gtcagacaggccccctgggaaggccctggagtgggtg gccgtgatctggtatgacgggagcaacaagtattac gcggattccgtcaaggggacgggttcaccatatcccg gataacagcaagaataactctttacttacagatgaac agcctgaggggccgaggacaccgcagtatattattgc gtaccaataatgactattgggggtcaggggcactctg gtgactgtgagcagc
Anti-PD1 (nVH9)	159	EVQLVQSGGGVVQPGRSLRL SCAASGITFSNSMHWVRQAP GKGLEWVAVI WYDGSKRYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDT AVYYCATNND YWGQGT LVT VSS	135	gagggtgcaactgggttcagtcgggcgggggctcgtc cagccggggcgagctctgcgcttgagctgtgctgcc tctgggattaccttttagcaactccatgcattgggtg cggcaggcaccgggaagggactggaatgggtcgca gtgatctggtacgatggatcaaagcgggtattacgcc gactccgtcaaaggccgggttcacaatcagccgcgac aacagcaaaaatactttatatcttcagatgaattcc cttagggcagaggatactgctgtgtattactgcgct actaacaacgattattgggggagggggacactagtc actgtttctagt
Anti-PD1 (nVH10)	160	EVQLVQSGGGVVQPGRSLRL SCAASGFTFSNSGMHWVRQA PGKGLEWVAVIWYDGSNKYY ADSVKGRFTISRDN SKNTLY LQMNSLRAEDTAVYYCATNN DYWGQGT LVT VSS	136	gagggtccagtttagtccaaagcggcggaggcgttagtg caacctggcagaagcctgcggttatcgtgcgccgca agcggcttcaccttttagcaattctggtatgcactgg gtcagacaggccccctgggaaggccctggagtgggtg gccgtgatctggtatgacgggagcaacaagtattac gcggattccgtcaaggggacgggttcaccatatcccg gataacagcaagaataactctttacttacagatgaac agcctgaggggccgaggacaccgcagtatattattgc gtaccaataatgactattgggggtcaggggcactctg gtgactgtgagcagc
Anti-PD1 (nVH11)	161	EVQLVQSGGGVVQPGRSLRL SCAASGFTFSYGMHWVRQA PGKGLEWVAVIWYDGSKYYA DSVKGRFTISRDN SKNTLYL QMNSLRAEDTAVYYCATNND YWGQGT LVT VSS	137	gagggtccagtttagtccaaagcggcggaggcgttagtg caacctggcagaagcctgcggttatcgtgcgccgca agcggcttcaccttttagctcttatggtatgcactgg gtcagacaggccccctgggaaggccctggagtgggtg gccgtgatctggtatgacgggagcaagtattacgcg gattccgtcaaggggacgggttcaccatatcccgcgat aacagcaagaataactctttacttacagatgaacagc ctgaggggccgaggacaccgcagtatattattgcgct accaataatgactattgggggtcaggggcactctgggtg actgtgagcagc

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
Anti-PD1 (nVH12)	162	QVQLVESGGGVVQPGRSLRL DCKASGITFSNSGMHWVRQA PGKGLEWVAVIWYDGSNKRY YADSVKGRFTISRDN SKNTL FLQMNSLRAEDTAVYYCATN NDYWGQGT LVTVSS	138	caggtgcagctggtggagtctgggggaggcgtggtc cagcctgggaggtccctgagactcgactgtaaagcg tctggaatcaccttcagtaactctggcatgcactgg gtccgccagggtccaggcaaggggctggagtgggtg gcagttatttggtatgatggaagtaacaaaagatac tatgcagactccgtgaagggccgattcaccatctcc agagacaattccaagaacacgctgtttctgcaaatg aacagcctgagagccgaggacacggctgtgtattac tgtgcgacaaacaacgactactggggccagggaacc ctggtcaccgtctcctca
Anti-PD1 (nVH12)	163	QVQLVESGGGVVQPGRSLRL DCKASGFTFSNSGMHWVRQA PGKGL	139	caggtgcagctggtggagtctgggggaggcgtggtc cagcctgggaggtccctgagactcgactgtaaagcg tctggattcaccttcagtaactctggcatgcactgg gtccgccagggtccaggcaaggggctggagtgggtg gcagttatttggtatgatggaagtaacaaaagatac tatgcagactccgtgaagggccgattcaccatctcc agagacaattccaagaacacgctgtttctgcaaatg aacagcctgagagccgaggacacggctgtgtattac tgtgcgacaaacaacgactactggggccagggaacc ctggtcaccgtctcctca
Anti-PD1 (nVH14)	164	EVQLVQSGGGVVQPGRSLRL SCAASGITFSNSGMHWVRQA PGKGLEWVAVIWYDGSKYYA DSVKGRFTISRDN SKNTLYL QMNSLRAEDTAVYYCATNND YWGQGT LVTVSS	140	gaggtgcaacttgtgcaaagcggcggcgagtcgtg cagcccggtcgatctcttcgcctgagttgtgctgcc agcggcattaccttttagcaattctggtatgcactgg gtacgtcaggcccccggtaaagggctagaatgggtg gctgtgatttggtacgatgggttctaagtactacgcc gacagcgtaaagggccgattcaccatcagtagagac aacagtaagaacaccctctacctccagatgaacagt ctgcgagctgaagacactgctgtgtactactgtgcc accaacaacgactactggggacagggaaccctggtc accgtgagtagt

Table 12 Exemplary anti-TGFβ VH and VL Sequences

Table 12 provides exemplary TGFβ receptor sequences for use in anti-PD1 fusion proteins described herein. In any embodiments exemplifying a fusion protein with, e.g., a TGFβRII ECD, an anti-TGFβ antibody (e.g., scFv, Fab), may be employed instead.

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
Anti-TGFb clone 1 VL	165	DIMMTQSPSS LAVSAGEKVT MSCKSSQS VL YSSNQKNYLA WYQQKPGQSP KLLIWASTR ESGVPDRFTG SGSGTDFTLT ISSVQAEDLA VYYCHQYLSS DTFGGGTKLE IKRTVA	179	gat ata atg atg aca cag agc ccc agc tct cta gct gtg agt gct ggc gag aag gtg acc atg agc tgt aag agc agt caa agc gtg ctg tac agt tcc aat cag aaa aat tac ctc gca tgg tat cag cag aag cca ggt caa agc cct aag ctc ctt atc tac tgg gcc tca acc cgt

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				gaa agt gga gtg cct gac aga ttt act ggt tca ggg agc ggc acc gat ttc act ctg act att agc tct gtg cag gca gaa gac ctt gcc gtg tat tac tgt cac cag tat ctg tct tca gac acg ttt gga ggt ggg acc aaa cta gaa atc aaa cgt act gtc gca
Anti-TGFb clone 1 VH	166	QVXLXQSGAE LVRPGTSVKV SCKASGYAFT NYLIEWVKQR PGQGLEWIGV NNPGSGGSNY NEKFKGKATL TADKSSSTAY MQLSSLTSD SAVYFCARSG GFYFDYWGQG TTQSPSPQPK RRAH	180	caggtgnnnctgnnncagagcggcgccgagctggtgag gcccggcaccagcgtgaagggtg agctgcaaggccagcggctacgccttcaccaactacct gatcgagtgggtgaagcagagg cccggccagggcctggagtggatcggcgtgaacaaccc cggcagcggcggcagcaactac aacgagaagttcaagggaaggccaccctgaccgccga caagagcagcagcaccgcctac atgcagctgagcagcctgaccagcgacgacagcgccgt gtacttctgcgccaggagcggc ggcttctacttcgactactggggccagggcaccacca gagccccagccccagcccaag aggaggggccac
Anti-TGFb clone 2 VL	167	DIQMTQSPSS LSASVGDRV ITCRASQSVL YSSNQKNYLA WYQQKPGKAP KLLIYWASTR ESGVPSRFSG SGSGTDFTLT ISSLPEDFA TYYCHQYLSS DTFGQGTKVE IKRTVA	181	gacatccagatgaccagagccccagcagcctgagcgc cagcgtgggcgacagggtgacc atcacctgcagggccagccagagcgtgctgtacagcag caaccagaagaactacctggcc tggtaccagcagaagcccggcaaggcccccaagctgct gatctactgggcccagcaccagg gagagcggcgtgcccagcaggttcagcggcagcggcag cggcaccgacttcaccctgacc atcagcagcctgcagcccaggacttcgccacctacta ctgccaccagtacctgagcagc gacaccttcggccagggcaccaaggtggagatcaagag gaccgtggcc
Anti-TGFb clone 2 VH	168	EVQLVESGGG LVQPGGSLRL SCAASGYAFT NYLIEWVRQA PGKGLEWVGV NNPGSGGSNY NEKFKGRATI SADNSKNTLY LQMNSLRAED TAVYYCARSG GFYFDYWGQG TLVTVSSAST KGPS	182	gaggtgcagctggtggagagcggcgggcggcctggtgca gcccggcggcagcctgaggctg agctgcgccgcccagcggctacgccttcaccaactacct gatcgagtgggtgaggcaggcc cccggcaaggcctggagtgggtgggcgtgaacaaccc cggcagcggcggcagcaactac aacgagaagttcaagggaaggccaccatcagcgccga caacagcaagaacaccctgtac ctgcagatgaacagcctgaggggccaggacaccgccgt gtactactgcgccaggagcggc ggcttctacttcgactactggggccagggcaccctggt gaccgtgagcagcgccagcacc aaggggccccagc
Anti-TGFb clone 3 VH	169	LARPGASVKM SCKTSGYTFT NYWMHWVRQR PGQGLEWIGT IYPGNSDTNY NQKFKDKAKL	183	ctggccaggccccggcgccagcgtgaagatgagctgcaa gaccagcggctacaccttcacc aactactggatgcactgggtgaggcagaggccccggcca gggcctggagtggatcggcacc atctacccccggcaacagcgacaccaactacaaccagaa gttcaaggacaaggccaagctg

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		TAVTSATTAY MELSSLTNED SAVYFCTRED SRSLYYNGWD YFDYWGQGTT LTVSS		accgccgtgaccagcgccaccaccgcctacatggagct gagcagcctgaccaacgaggac agcgccgtgtacttctgcaccagggaggacagcaggag cctgtactacaacggctgggac tacttcgactactggggccagggcaccaccctgaccgt gagcagc
Anti-TGFb clone 3 VL	170	LTQSPASLAV SLGQRATISC RASESVDNYG ISFLNWFQK PGQPPKLLIY AASNQGSQVP ARFSGSGSGT DFSLNIHPME EDDTGMYFCQ QSKEVPRTFG GGTKLEII	184	ctgacccagagccccgccagcctggccgtgagcctggg ccagagggccaccatcagctgc agggccagcgagagcgtggacaactacggcatcagctt cctgaactggttccagcagaag cccggccagccccccaagctgctgatctacgccgccag caaccagggcagcggcgtgccc gccaggttcagcggcagcggcagcggcaccgacttcag cctgaacatccaccccatggag gaggacgacaccggcatgtacttctgccagcagagcaa ggaggtgcccaggaccttcggc ggcggcaccaagctggagatcatc
Anti-TGFb clone 4 VH	171	QVQLVQSGAEVKKPGSSVKV SCKASGGTFSSYAISWVRQA PGQGLEWMGGIIPFGTAN YAQKFQGRVTITADESTSTA YMESSLRSEDYAVYYCARG LWEVRALPSVYWGQGLVTV SS	185	caggtgcagctggtgcagagcggcgccgaggtgaagaa gcccggcagcagcgtgaagggtg agctgcaaggccagcggcgccaccttcagcagctacgc catcagctgggtgaggcaggcc cccggccagggcctggagtggatggcgccatcatccc catcttcggcaccgcccaactac gcccagaagttccagggcagggtgaccatcaccgccga cgagagcaccagcaccgcctac atggagctgagcagcctgaggagcaggacaccgccgt gtactactgcgccaggggcctg tgggaggtgagggccctgcccagcgtgtactggggcca gggcaccctggtgaccgtgagc agc
Anti-TGFb clone 4 VL	172	SYELTQPPSVSVAPGQTARI TCGANDIGSKSVHWYQQKAG QAPVLVSEDIIRPSGIPER I SGSNSGNTATLTISRVEAGD EADYYCQVWDRSDQYVFGT GTKVTVLG	186	agctacgagctgaccagccccccagcgtgagcgtggc ccccggccagaccgccaggatc acctgcggcgccaacgacatcggcagcaagagcgtgca ctggtaccagcagaaggccggc caggccccctgctggtggtgagcaggacatcatcag gcccagcggcatccccgagagg atcagcggcagcaacagcggcaacaccgccaccctgac catcagcagggtggaggccggc gacgaggccgactactactgccaggtgtgggacaggga cagcgaccagtacgtgttcggc accggcaccaaggtgaccgtgctgggc
Anti-TGFb clone 5 VH	173	QVQLVQSGAE VKKPGSSVKV SCKASGYTFS SNVISWVRQA PGQGLEWMGG VIPIVDIANY AQRFKGRVTI TADESTSTTY MELSSLRSED TAVYYCASTL GLVLDAMDYW	187	caggtgcagctggtgcagagcggcgccgaggtgaagaa gcccggcagcagcgtgaagggtg agctgcaaggccagcggctacaccttcagcagcaacgt gatcagctgggtgaggcaggcc cccggccagggcctggagtggatggcgccgctgatccc catcgtggacatcgccaactac gcccagaggttcaagggcagggtgaccatcaccgccga cgagagcaccagcaccacctac atggagctgagcagcctgaggagcaggacaccgccgt gtactactgcgccagcaccctg ggcctggtgctggacgccatggactactggggccaggg

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		GQGTLLTVSS		caccctggtgaccgtgagcagc
Anti-TGFb clone 5 VL	174	ETVLTQSPGT LSLSPGERAT LSCRASQSLG SSYLAWYQQK PGQAPRLLIY GASSRAPGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYADSPITFG QGTRLEIK	188	gagaccgtgctgacccagagccccggcaccctgagcct gagccccggcgagagggccacc ctgagctgcagggccagccagagcctgggcagcagcta cctggcctggtaccagcagaag cccgccaggccccaggctgctgatctacggcgccag cagcagggccccggcatcccc gacaggttcagcggcagcggcagcggcaccgacttcac cctgaccatcagcaggctggag cccaggacttcgccgtgtactactgccagcagtacgc cgacagccccatcaccttcggc cagggcaccaggctggagatcaag
Anti-TGFb clone 6 VH	175	QVQLVQSGAE VKKPGSSVKV SCKASGYTFS SNVISWVRQA PGQGLEWMGG VIPIVDIANY AQRFKGRVTI TADESTSTTY MELSSLRSED TAVYYCALPR AFVLDAMDYW GQGTLLTVSS	189	caggtgcagctggtgcagagcggcgccgaggtgaagaa gccccggcagcagcgtgaaggtg agctgcaaggccagcggctacaccttcagcagcaacgt gatcagctgggtgaggcaggcc cccgccaggccctggagtggatggcgccgtgatccc catcgtggacatcgccaactac gcccagaggttcaagggcagggtgaccatcacccgccga cgagagcaccagcaccacctac atggagctgagcagcctgaggagcaggacaccgccgt gtactactgcgccctgccagg gccttcgtgctggacgccatggactactggggccaggg caccctggtgaccgtgagcagc
Anti-TGFb clone 6 VL	176	ETVLTQSPGT LSLSPGERAT LSCRASQSLG SSYLAWYQQK PGQAPRLLIY GASSRAPGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYADSPITFG QGTRLEIK	190	gagaccgtgctgacccagagccccggcaccctgagcct gagccccggcgagagggccacc ctgagctgcagggccagccagagcctgggcagcagcta cctggcctggtaccagcagaag cccgccaggccccaggctgctgatctacggcgccag cagcagggccccggcatcccc gacaggttcagcggcagcggcagcggcaccgacttcac cctgaccatcagcaggctggag cccaggacttcgccgtgtactactgccagcagtacgc cgacagccccatcaccttcggc cagggcaccaggctggagatcaag
Anti-TGFb clone 7 VH	177	QVQLVQSGAE VKKPGSSVKV SCKASGGTFS TSFINWVRQA PGQGLEWMGG IIPFDITNY AQKFQSRVTI TADKSTSTAY MELSSLRSED TAVYYCARGN GNYALDAMDY WGQGTLLTVS S	191	caggtgcagctggtgcagagcggcgccgaggtgaagaa gccccggcagcagcgtgaaggtg agctgcaaggccagcggcgccaccttcagcaccagctt catcaactgggtgaggcaggcc cccgccaggccctggagtggatggcgcccatcatccc catcttcgacatcaccaactac gcccagaagttccagagcagggtgaccatcacccgccga caagagcaccagcaccgcctac atggagctgagcagcctgaggagcaggacaccgccgt gtactactgcgccaggggcaac ggcaactacgccctggacgccatggactactggggcca gggcaccctggtgaccgtgagc agc
Anti-TGFb	178	EIVLTQSPGT LSLSPGERAT	192	gagatcgtgctgacccagagccccggcaccctgagcct gagccccggcgagagggccacc

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
clone 7 VL		LSCRASQSVS SSYFAWYQQK PGQAPRLLIY GASSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYYDSPITFG QGTRLEIK		ctgagctgcagggccagccagagcgtgagcagcagcta cttcgcctggtaccagcagaag cccgggccaggccccaggctgctgatctacggcgccag cagcaggggccaccggcatcccc gacaggttcagcggcagcggcagcggcaccgacttcac cctgaccatcagcaggctggag cccgaggacttcgccgtgtactactgccagcagtacta cgacagccccatcaccttcggc cagggcaccaggctggagatcaag

Table 13 Exemplary TGFβ1 Inhibitory Peptide and Nucleotide Sequences

Table 13 provides exemplary TGFβ1 inhibitory peptides for use in anti-PD1 fusion proteins described herein. In any embodiments exemplifying a fusion protein with, e.g., a TGFβRII ECD, a TGFβ1 inhibitory peptides may be employed instead.

Name	SEQ ID NO	Amino acid sequence	SEQ ID NO	Nucleotide sequence
TGFb1-I36	263	HANFCLGPCPYI WSLA	268	CACGCCAACTTCTGCCTGGGCCCCTGCCCCTACAT CTGGAGCCTGGCC
TGFb1-I37	264	FCLGPCPYIWSL DTA	269	TTCTGCCTGGGCCCCTGCCCCTACATCTGGAGCCT GGACACCGCC
TGFb1-I38	265	SNPYSAFQVDII VDIA	270	AGCAACCCCTACAGCGCCTTCCAGGTGGACATCA TCGTGGACATCGCC
TGFb1-I39	266	TSLDATMIWTM MA	271	ACCAGCCTGGACGCCACCATGATCTGGACCATGA TGGCC
TGFb1-I40	267	TSLDASIWAMM QNA	272	ACCAGCCTGGACGCCAGCATCTGGGCCATGATGC AGAACGCC

Table 14. Exemplary ADA2 Sequences

Table 14 provides exemplary ADA2 sequences for use in anti-PD1 fusion proteins described herein. In any embodiments exemplifying a fusion protein with, e.g., a TGFβRII ECD, an ADA2 sequence may be employed instead.

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
ADA2 mutant 1	273	IDETRAHLLLKEKMMRLGGR LVLNTKEELANERLMTLKIA EMKEAMRTLIFPPSMHFFQA KHLIERSQVFNI LRMMPKGA ALHLHDIGIVTMDWLVRNVT YRPHGIALPGDSL LRNFTLV TQHPEVIYTNQNVVLSKFET IFFTISGLIHYAPVFRDYVF RSMQEFYEDNVLYMEIRASL LPVYELSGEHHDEEWSVKTY QEVAQDFVETHPEFIGIKII YSDHRSKDVA VIAESIRMAM GLRIKFPTVVAGFDLVGHED		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		TGHSLHDYKEALMIPAKDGV KLPYFFHAGETDWQGTSIDR NILDALMLNTTRIGHGFALS KHPAVRTYSWKKDIPIEVCP ISNQVLKLVSDLRNHPVATL MATGHPMVISSDDPAMFGAK GLSYDFYEVFMGIGGMKADL RTLKQLAMNSIKYSTLLESE KNTFMEIWKKRWDKFIADVA TK		
ADA2 mutant 2	274	IDETRAHLLLKEKMMRLGGR LVLNTKEELANERLMTLKIA EMKEAMRTLIFPPSMHFFQA KHLIERSQVFNILRMMPKGA ALHLHDIGIVTMDWLVRNVT YRPHGIALPGDSLRLNFTLV TQHPEVIYTNQNVVWSKFET IVFTISGLIHYAPVFRDYVF RSMQEFYEDNVLYMEIRARL LPVYELSGEHHDEEWSVKTY QEVAQDFVETHPEFIGIKII YSDHRSKDVAVIAESIRMAM GLRIKFPTVVAGFDLAGHED TGHSLHDYKEALMIPAKDGV KLPYFFHAGETDWQGTSIDR NILDALMLNTTRIGHGFALS KHPAVRTYSWKKDIPIEVCP ISNQVLKLVSDLRNHPVATL MATGHPMVISSDDPAMFGAK GLSYDFYEVFMGIGGMKADL RTLKQLAMNSIKYSTLLESE KNTFMEIWKKRWDKFIADVA TK		
ADA2 mutant 3	275	EMKEAMRTLIFPPSMHFFQA KHLIERSQVFNILRMMPKGA ALHLHDIGIVTMDWLVRNVT YRPHGIALPGDSLRLNFTLV TQHPEVIYTNQNVVLSKFET IVFTISGLIHYAPVFRDYVF RSMQEFYEDNVLYMEIRARL LPVYELSGEHHDEEWSVKTY QEVAQDFVETHPEFIGIKII YSDHRSKDVAVIAESIRMAM GLRIKFPTVVAGFDLVGHED TGHSLHDYKEALMIPAKDGV KLPYFFHAGETDWQGTSIDR NILDALMLNTTRIGHGFALS KHPAVRTYSWKKDIPIEVCP ISNQVLKLVSDLRNHPVATL MATGHPMVISSDDPAMFGAK GLSYDFYEVFMGIGGMKADL RTLKQLAMNSIKYSTLLESE KNTFMEIWKKRWDKFIADVA TK		
ADA2 mutant 4	276	IDETRAHLLLKEKMMRLGGR LVLNTKEELANERLMTLKIA EMKEAMRTLIFPPSMHFFQA		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		KHLIERSQVFNI LRMPKGA ALHLHDIGIVTMDWLGGGGS GGGGSVTEFDDSLRNFTLV TQHPEVIYTNQNVVWSKFET IFFTISGLIHYAPVFRDYVF RSMQEFYEDNVLYMEIRARL LPVYELSGEHHDEEWSVKTY QEVAQEFVETHPEFIGIKII YSDHRSRDVAVIAESIRMAM GLRIKFPTVVAGFDLSGHED TGHSLHDYKEALMIPAKDGV KLPYFFHAGETDWQGTSIDR NILDALMLNTTRIGHGFALS KHPAVRTYSWKKDPIEVCP ISNQVLKLVSDLRNHPVATL MATGHPMVISSDDPAMFGAK GLSYDFYEVFMGIGGMKADL RTLKQLAMNSIKYSTLLESE KNTFMEIWKKRWKFIADVA TK		
ADA2 mutant 5	277	IDETRAHLLLKEKMMRLGGR LVLNTKEELANERLMTLKIA EMKEAMRTLIFPPSMHFFQA KHLIERSQVFNI LRMPKGA ALHLHNIGIVTMDWLGGGGS GGGGSVTEFDDSLRNFTLV TQHPEVIYTNQNVVWSKFET IFFTISGLIHYAPVFRDYVF RSMQEFYEDNVLYMEIRARL LPVYELSGEHHDEEWSVKTY QEVAQDFVETHPEFIGIKII YSDHRSYDVAVIAESIRMAM GLRIKFPTVVAGFDLVGHED TGHSLHDYKEALMIPAKDGV KLPYFFHAGETDWQGTSIDR NILDALMLNTTRIGHGFALS KHPAVRTYSWKKDPIEVCP ISNQVLKLVSDLRNHPVATL MATGHPMVISSDDPAMFGAK GLSYDFYEVFMGIGGMKADL RTLKQLAMNSIKYSTLLESE KNTFMEIWKKRWKFIADVA TK		
ADA2 mutant 6	278	IDETRAHLLLKEKMMRLGGR LVLNTKEELANERLMTLKIA EMKEAMRTLIFPPSMHFFQA KHLIERSQVFNI LRMPKGA ALHLHDIGIVTMDWLVRNVT YRPHCHICFTPRGIMQFRFA HPTPRPSEKCSKWILLEDYR KRVQNVTEFDDSLRNFTLV TQHPEVIYTNQNVVWSKFET IFFTISGLIHYAPVFRDYVF RSMQEFYEDNVLYMEIRARL LPVYELSGEHHDEEWSVKTY QEVAQKFVETHPEFIGIKII YSDHRSKDVAVIAESIRMAM		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		GLRIKFPTVVAGFDLVGHED TGHSLHDYKEALMIPAKDGV KLPYFFHAGETDWQGTSIDR NILDALMLNTTRIGHGFALS KHPAVRTYSWKKDIPIEVCP ISNQVLKLVSDLRNHPVATL MATGHPMVISSDDPAMFGAK GLSYDFYEVFMGIGGMKADL RTLKQLAMNSIKYSTLLESE KNTFMEIWKKRWDFIADVA TK		
ADA2 mutant 7	279	IDETRAHLLLKEKMMRLGGR LVLNTKEELANERLMTLKIA EMKEAMRTLIFPPSMHFFQA KHLIERSQVFNI LRMPKGA ALHLHDIGIVTMDWLVRNVT YRPHCHICFTPRGIMQFRFA HPTPRPSEKCSKWILLEDYR KRVQNVTEFDDSLRNFTLV TQHPEVIYTNQNVVWSKFET IFFTISGLIHYAPVFRDYVF RSMQEFYEDNVLYMEIRAQL LPVYELSGEHHDEEWSVKTY QEVAKFVETHPEFIGIKII YNDHRSKDVAVIAESIRMAM GLRIKFPTVVAGFDLVGHED TGHSLHDYKEALMIPAKDGV KLPYFFHAGETDWQGTSIDR NILDALMLNTTRIGHGFALS KHPAVRTYSWKKDIPIEVCP ISNQVLKLVSDLRNHPVATL MATGHPMVISSDDPAMFGAK GLSYDFYEVFMGIGGMKADL RTLKQLAMNSIKYSTLLESE KNTFMEIWKKRWDFIADVA TK		
VH6 ADA2	280	QVQLVESGGGVVQGRSLRL DCKASGITFSNSGMHWVRQA PGKGLEWVAV IWYDGSKRYYADSVKGRFTI SRDNSKNTLFLQMNSLRAED TAVYYCATND DYWGQGTLLTVSSASTKGPS VFPLAPCSRSTSESTAALGC LVKDYFPEPV TVSWNSGALTSGVHTFPAVL QSSGLYSLSSVTVPSSSLG TKTYTCNVDH KPSNTKVDKRVESKYGPSCP PCPAPEFLGGPSVFLFPPKP KDTLMISRTP EVTCTVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFN STYRVVSVLT VLHQDWLNGKEYKCKVSNKG LPSSIEKTISKAKGQPREPQ VYTLPPSQEE	285	CAGGTGCAGCTGGTCGAAAGCGGAGGAGGAGTGGTCCAG CCAGGACGATCCCTGAGACTGGATTGTAAGGCCTCTGGA ATCACATTCTCTAACAGTGGAATGCACTGGGTGCGCCAG GCACCAGGAAAAGGACTGGAGTGGGTGGCCGTCATCTGG TACGACGGGTCAAAGCGATACTATGCAGATAGCGTGAAA GGAAGGTTTACAATTTACGCGACAACAGCAAGAATACT CTGTTTCTGCAGATGAACCTCTCTGAGAGCAGAGGATACT GCCGTGTACTATTGTGCTACCAATGACGATTATTGGGGG CAGGGAACCTCTGGTGACCGTCAGTTCAGCTAGCACCAAG GGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGC ACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCA GGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTC CTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTG ACCGTGCCCTCCAGCAGCTTGGGCACGAAGACCTACACC TGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGAC AAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCACCA TGCCCAGCACCTGAGTTCCTGGGGGGACCATCAGTCTTC CTGTTCCCCCCCCAAAACCAAGGACACTCTCATGATCTCC CGGACCCCTGAGGTACGTCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGAT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		MTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTPPV LDSDGSFFLY SRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSPGK GGGSGGGGS IDETRAHLLLKEKMMRLGGR LVLNTKEELANERLMTLKIA EMKEAMRTLI FPPSMHFFQAKHLIERSQVF NILRMMPKGAALHLHDIGIV TMDWLVRNVT YRPHCHICFTPRGIMQFRFA HPTPRPSEKCSKWILLEDYR KRVQNVTEFD DSLLRNFTLVTQHPEVIYTN QNVVWSKFETIFFTISGLIH YAPVFRDYVF RSMQEFYEDNVLYMEIRARL LPVYELSGEHHDEEWSVKTY QEVAQKFVET HPEFIGIKIISDHRSDVA VIAESIRMAMGLRIKFPTVV AGFDLVGHED TGHSLHDYKEALMIPAKDGV KLPYFFHAGETDWQGSIDR NILDALMLNT TRIGHGFALSKHPAVRTYSW KKDIPIEVCPISNQVLKLVS DLRNHPVATL MATGHPMVISSDDPAMFGAK GLSYDFYEVFMGIGGMKADL RTLKQLAMNS IKYSTLLESEKNTFMEIWKK RWDKFIADVATK		GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAG GAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCCTC ACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTAC AAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCTCCATC GAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAG CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATG ACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGC TTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT GGGCAGCCGGAGAACAACATAAGACCACGCCTCCCGTG CTGGACTCCGACGGCTCCTTCTTCTCTACAGCAGGCTC ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTC TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTAC ACACAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGGTGGA GGTGGTTCTGGAGGTGGAGGTAGTATCGACGAAACCAGA GCACACTTACTGCTGAAAGAGAAAATGATGCGCCTGGGC GGGAGATTGGTGTAAATACTAAGGAAGAGCTGGCAAAT GAAAGACTCATGACACTGAAGATTGCTGAAATGAAGGAG GCGATGAGGACGCTGATCTTTCGCGCTTCCATGCACTTC TTCCAAGCTAAACACCTGATCGAAAGATCCCAAGTGTTT AACATCCTGAGGATGATGCCTAAGGGGGCCGCTCTGCAC CTTCACGATATTGGGATTGTAACAATGGACTGGCTGGTA AGGAACGTGACATACAGACCTCATTGCCATATTTGTTTT ACTCCCCGAGGAATCATGCAATTCAGGTTTGCCCAACCA ACTCCTCGGCCAAGCGAGAAGTGTAAGTGGATTTTG CTGGAAGATTACCGTAAGCGCGTGCAGAATGTGACAGAG TTTGATGACTCCCTGCTCCGCAATTTTACCCTGGTGACC CAGCACCCCGAAGTTATATACATAACCAAAATGTCGTG TGGTCCAAGTTTGAGACGATCTTCTTCACGATTTACAGGC TTGATCCACTACGCCCCGGTCTTTCGGGATTATGTGTTT AGGAGTATGCAGGAGTTTTATGAGGATAATGTTCTGTAC ATGGAGATCCGAGCCCGGCTGCTTCCAGTCTACGAACTA TCCGGCGAACACCATGACGAGGAATGGAGCGTCAAGACC TATCAAGAGGTGGCCCAGAAGTTCGTAGAAACGCATCCA GAGTTCATCGGTATTAAGATTATCTACTCTGATCACCGC TCAAAGGATGTGGCTGTCATCGCCGAGTCTATACGGATG GCCATGGGCCTGCGGATTAAGTTCCTACCGTCGTCGCC GGATTCGACCTCGTTGGGCATGAGGATACTGGCCATAGT CTCCATGACTATAAAGAAGCCCTTATGATCCCAGCAAAG GACGGAGTGAAGCTGCCCTACTTCTTCCACGCAGGGGAG ACCGACTGGCAGGGAACGAGCATCGACCGGAACATACTT GATGCACTCATGCTTAATACCACACGAATCGGCCACGGC TTCGCTCTCTCCAAGCACCCAGCCGTGAGAACCTACAGC TGGAAGAAGGATATCCCCATCGAGGTTTGTCCCATCAGC AATCAGGTGCTGAAATTGGTGAGTGACCTGAGAAACCAC CCAGTCGCAACATTAATGGCCACTGGCCACCCTATGGTG ATTTCAAGCGATGATCCAGCCATGTTCCGAGCAAAAGGA CTCAGTTACGACTTCTATGAGGTATTCATGGGTATTGGT GGTATGAAGGCAGACCTGCGGACTCTTAAGCAGTTGGCA ATGAACTCAATTAAGTACTCTACCTTATTGGAGTCTGAA AAGAACACATTTATGGAGATCTGGAAAAAGCGCTGGGAC AAATTCATCGCAGATGTTGCCACAAAA
VH6 ADA2 mut 7	281	QVQLVESGGGVVQPGRSLRL DCKASGITFSNSGMHWVRQA PGKGLEWVAV IWYDGSKRYYADSVKGRFTI SRDNSKNTLFLQMNSLRAED TAVYYCATND	286	CAGGTGCAGCTGGTCGAAAGCGGAGGAGGAGTGGTCCAG CCAGGACGATCCCTGAGACTGGATTGTAAGGCCTCTGGA ATCACATTCTCTAACAGTGGAATGCACTGGGTGCGCCAG GCACCAGGAAAAGGACTGGAGTGGGTGGCCGTCATCTGG TACGACGGGTCAAAGCGATACTATGCAGATAGCGTGAAA GGAAGGTTCACAATTTACGCGACAACAGCAAGAATACT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		DYWGQGTLLTVSSASTKGPS VFPLAPCSRSTSESTAALGC LVKDYFPEPV TVSWNSGALTSGVHTFPAVL QSSGLYSLSSVTVPSSSLG TKTYTCNVDH KPSNTKVDKRVESKYGPPCP PCPAPEFLGGPSVFLFPPKP KDTLMIS RTP EVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFN STYRVVSVLT VLHQDWLNGKEYKCKVSNKG LPSSIEKTISKAKGQPREPQ VYTLPPSQEE MTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPV LDSDGSFFLY SRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSPGK GGGGSGGGGS IDETRAHLLLKEKMMRLGGR LVLNTKEELANERLMTLKIA EMKEAMRTLI FPPSMHFFQAKHLIERSQVF NILRMMPKGAALHLHDIGIV TMDWLVRNVT YRPHCHICFTPRGIMQFRFA HPTPRPSEKCSKWILLEDYR KRVQNVTEFD DSLLRNFTLVTQHPEVIYTN QNVVWSKFETIFFTISGLIH YAPVFRDYVF RSMQEFYEDNVLYMEIRAQL LPVYELSGEHHDEEWSVKTY QEVAQKFVET HPEFIGIKIIYNDHRSKDVA VIAESIRMAMGLRIKFPTVV AGFDLVGHED TGHS LH DYKEALMIPAKDGV KLPYFFHAGETDWQGSIDR NILDALMLNT TRIGHGFALSKHPAVRTYSW DKDIPIEVCPI SNQVLKLVS DLRNHPVATL MATGHMVIS SDDPAMFGAK GLSYDFYEVFMGIGGMKADL RTLKQLAMNS IKYSTLLESEKNTFMEIWKK RWDKFIADVATK		CTGTTTCTGCAGATGAACTCTCTGAGAGCAGAGGATACT GCCGTGTACTATTGTGCTACCAATGACGATTATTGGGGG CAGGGAACCTCTGGTGACCGTCAGTTCAGCTAGCACCAAG GGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGC ACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCA GGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTC CTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTG ACCGTGCCCTCCAGCAGCTTGGGCACGAAGACCTACACC TGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGAC AAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCACCA TGCCCAGCACCTGAGTTCCTGGGGGGACCATCAGTCTTC CTGTTCCCCC AAAACCCAAGGACACTCTCATGATCTCC CGGACCCCTGAGGT CACGTGCGTGGTGGTGGACGTGAGC CAGGAAGACCCCGAGGTCCAGTTC AACTGGTACGTGGAT GCGGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAG GAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCCTC ACCGTCTGCACCAGGACTGGCTGAACGGCAAGGAGTAC AAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCTCCATC GAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAG CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATG ACCAAGAACCAGGT CAGCCTGACCTGCCTGGTCAAAGGC TTCTACCC CAGCGACATCGCCGTGGAGTGGGAGAGCAAT GGGCAGCCGGAGAACA ACTACAAGACCACGCCTCCCGTG CTGGACTCCGACGGCTCCTTCTTCTCTACAGCAGGCTC ACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTC TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTAC ACACAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGGTGGA GGTGGTTCTGGAGGTGGAGGTAGTATCGACGAAACCAGA GCACACTTACTGCTGAAAGAGAAAATGATGCGCCTGGGC GGGAGATTGGTGTTAAATACTAAGGAAGAGCTGGCAAAT GAAAGACTCATGACACTGAAGATTGCTGAAATGAAGGAG GCGATGAGGACGTGATCTTTCGCGCTTCCATGCACTTC TTCCAAGCTAAACACCTGATCGAAAGATCCCAAGTGTTT AACATCCTGAGGATGATGCCTAAGGGGGCCGCTCTGCAC CTTACGATATTGGGATTGTAACAATGGACTGGCTGGTA AGGAACGTGACATACAGACCTCATTGCCATATTTGTTTT ACTCCCCGAGGAATCATGCAATTCAGGTTTGCCACCCA ACTCCTCGGCCAAGCGAGAAGTG TAGTAAGTGGATTTTG CTGGAAGATTACCGTAAGCGCGTGCAGAATGTGACAGAG TTTGATGACTCCCTGCTCCGCAATTTTACCCTGGTGACC CAGCACCCCGAAGTTATATACTAACC AAAATGTCGTG TGGTCCAAGTTTGAGACGATCTTCTTACGATTT CAGGC TTGATCCACTACGCCCCGGTCTTTCGGGATTATGTGTTT AGGAGTATGCAGGAGTTTTATGAGGATAATGTTCTGTAC ATGGAGATCCGAGCCCAGCTGCTTCCAGTCTACGAACTA TCCGGCGAACACCATGACGAGGAATGGAGCGTCAAGACC TATCAAGAGGTGGCCCAGAAGTTCGTAGAAACGCATCCA GAGTTCATCGGTATTAAGATTATCTACAATGATCACCGC TCAAAGGATGTGGCTGTCATCGCCGAGTCTATACGGATG GCCATGGGCCTGCGGATTAAGTTCCCTACCGTCGTCGCC GGATTCGACCTCGTTGGGCATGAGGATACTGGCCATAGT CTCCATGACTATAAAGAAGCCCTTATGATCC CAGCAAAG GACGGAGTGAAGCTGCCCTACTTCTTCCACGCAGGGGAG ACCGACTGGCAGGGAACGAGCATCGACCGGAACATACTT GATGCACTCATGCTTAATACCACACGAATCGGCCACGGC TTCGCTCTCTCCAAGCACCCAGCCGTGAGAACCTACAGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				TGGGATAAGGATATCCCCATCGAGGTTTGTCCCATCAGC AATCAGGTGCTGAAATTGGTGAGTGACCTGAGAAACCAC CCAGTCGCAACATTAATGGCCACTGGCCACCCTATGGTG ATTTCAAGCGATGATCCAGCCATGTTCTGGAGCAAAAGGA CTCAGTTACGACTTCTATGAGGTATTCATGGGTATTGGT GGTATGAAGGCAGACCTGCGGACTCTTAAGCAGTTGGCA ATGAACTCAATTAAGTACTCTACCTTATTGGAGTCTGAA AAGAACACATTTATGGAGATCTGGAAAAAGCGCTGGGAC AAATTCATCGCAGATGTTGCCACAAAA
VH7 ADA2	282	QVQLVQSGVEVKKPGASVKV SCKASGYTFTNYYMYWVRQA PGQGLEWMGG INPSNGGTNFNEKFKNRVT LTTDSSTTTAYMELKSLQFDD TAVYYCARRD YRFDMGFDYWGGQTTVTVSS ASTKGPSVFPLAPCSRSTSE STAALGCLVK DYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVT VPSSSLGTKT YTCNVDPKPSNTKVDKRVES KYGPPCPPCPAPEFLGGPSV FLFPPKPKDT LMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTK PREEQFNSTY RVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTIKAK GQPREPQVY TLPDSQSEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENN YKTTTPVLD SDSGSFLYSLRTVDSRWQEG NVFSCSVMEALHNHYTQKS LSLSPGKGGG GSGGGGSDIDETRAHLLLEK MMRLGGRLVLNTKEELANER LMTLKIAEMK EAMRTLIFPPSMHFFQAKHL IERSQVFNIILRMMPKGAALH LHDIGIVTMD WLVNRNVYRPHCHICFTPRG IMQFRFAHPTPRPSEKCSKW ILLEDYRKR VQNVTEFDDSLLRNFTLVTQH PEVIYTNQNVVWSKFETIFF TISGLIHYAP VFRDYVFRSMQEFYEDNVLY MEIRARLLPVYELSGEHHDE EWSVKTYQEV AQKFVETHPEFIGIKIIYSD HRSKDVAIVIAESIRMAMGLR IKFPTVVAGF DLVGHEDTGHSLHDYKEALM IPAKDGVKLPYFFHAGETDW QGTSIDRNIL	287	CAGGTGCAGCTGGTCCAGAGCGGCGTGGAAGTCAAGAAA CCCGGGGCTCAGTGAAGGTCAGCTGTAAAGCTTCCGGC TACACCTTCACAACTACTATATGTATTGGGTGAGACAG GCACCAGGACAGGGACTGGAGTGGATGGGCGGGATTAA CCTAGTAATGGAGGCACTAACTTCAACGAAAAGTTTAA AACAGGGTGACCCTGACCACAGATTCAAGCACTACCACA GCTTACATGGAGCTGAAGTCCCTGCAGTTTGACGATACA GCCGTGTACTATTGTGCTCGGAGAGACTACAGGTTTCGAT ATGGGCTTTGACTATTGGGGCCAGGGGACTACCGTGACC GTCTCCTCTGCTAGCACCAAGGGCCCATCGGTCTTCCCC CTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCC GCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG GTGACGGTGTCTGTGGAAGTCAAGGCGCCCTGACCAGCGG GTGCACACCTTCCCGGCTGTCTACAGTCTCAGGACTC TACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGC TTGGGCACGAAGACCTACACCTGCAACGTAGATCACAAG CCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAA TATGGTCCCCCATGCCCACCATGCCCAGCACCTGAGTTC CTGGGGGGGACCATCAGTCTTCTGTTCCCCCAAAACCC AAGGACACTCTCATGATCTCCCGGACCCCTGAGGTACAG TGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTC CAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAAT GCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACG TACCGTGTGGTCAAGCTCCTCACCCTGTCACCAGGAC TGGCTGAACGGCAAGGAGTACAAGTGAAGGTCTCCAAC AAAGGCCTCCCGTCTCCATCGAGAAAACCATCTCCAAA GCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTG CCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTACAGC CTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC TACAAGACCACGCCTCCCGTGTGCTGACTCCGACGGCTCC TTCTTCTCTACAGCAGGCTCACCCTGGACAAGAGCAGG TGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCAT GAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCC CTGTCTCCGGGTAAAGGTGGAGGTGGTTCTGGAGGTGGA GGTAGTATCGACGAAACCAGAGCACACTTACTGCTGAAA GAGAAAATGATGCGCCTGGGCGGGAGATTGGTGTTAAAT ACTAAGGAAGAGCTGGCAAATGAAAGACTCATGACACTG AAGATTGCTGAAATGAAGGAGGCGATGAGGACGCTGATC TTTCCGCCTTCCATGCACTTCTTCCAAGCTAAACACCTG ATCGAAAGATCCCAAGTGTAAACATCCTGAGGATGATG CCTAAGGGGGCCGCTCTGCACCTTCACGATATTGGGATT GTAACAATGGACTGGCTGGTAAGGAACGTGACATACAGA CCTCATTGCCATATTTGTTTTACTCCCCGAGGAATCATG CAATTCAGGTTTGCCCAACCACTCCTCGGCCAAGCGAG AAGTGATGTAAGTGGATTTTGCTGGAAGATTACCGTAAG CGCGTGCAGAATGTGACAGAGTTTGATGACTCCCTGCTC CGCAATTTTACCCTGGTGACCCAGCACCCCGAAGTTATA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		DALMLNTRIGHGFALSKHP AVRTYSWKKDIPIEVCPISN QVLKLVSDLR NHPVATLMATGHPMVISSDD PAMFGAKGLSYDFYEVFMGI GGMKADLRTL KQLAMNSIKYSTLLESEKNT FMEIWKKRWDKFIADVATK		TACACTAACCAAAATGTCGTGTGGTCCAAGTTTGAGACG ATCTTCTTCACGATTTTCAAGGCTTGATCCACTACGCCCCG GTCTTTCGGGATTATGTGTTTAGGAGTATGCAGGAGTTT TATGAGGATAATGTTCTGTACATGGAGATCCGAGCCCCG CTGCTTCCAGTCTACGAACTATCCGGCGAACACCATGAC GAGGAATGGAGCGTCAAGACCTATCAAGAGGTGGCCCAG AAGTTCGTAGAAACGCATCCAGAGTTCATCGGTATTAAG ATTATCTACTCTGATCACCGCTCAAAGGATGTGGCTGTC ATCGCCGAGTCTATACGGATGGCCATGGGCCTGCGGATT AAGTTCCTACCGTCGTCGCCGATTTCGACCTCGTTGGG CATGAGGATACTGGCCATAGTCTCCATGACTATAAAGAA GCCCTTATGATCCCAGCAAAGGACGGAGTGAAGCTGCCC TACTTCTTCACGCAGGGGAGACCGACTGGCAGGGAACG AGCATCGACCGGAACATACTTGATGCACTCATGCTTAAT ACCACACGAATCGGCCACGGCTTCGCTCTCTCCAAGCAC CCAGCCGTGAGAACCTACAGCTGGAAGAAGGATATCCCC ATCGAGGTTTGTCCCATCAGCAATCAGGTGCTGAAATTG GTGAGTGACCTGAGAAACCACCCAGTCGCAACATTAATG GCCACTGGCCACCCTATGGTGATTTCAAGCGATGATCCA GCCATGTTTCGGAGCAAAGGACTCAGTTACGACTTCTAT GAGGTATTCATGGGTATTGGTGGTATGAAGGCAGACCTG CGGACTCTTAAGCAGTTGGCAATGAACTCAATTAAGTAC TCTACCTTATTGGAGTCTGAAAAGAACACATTTATGGAG ATCTGGAAAAAGCGCTGGGACAAATTCATCGCAGATGTT GCCACAAAA
VH7 ADA2 mut7	283	QVQLVQSGVEVKKPGASVKV SCKASGYFTNYMYWVRQA PGQGLEWMGG INPSNGGTNFNEKFKNRVTL TTDSSTTTAYMELKSLQFDD TAVYYCARRD YRFDMGFDYWQGTTVTVSS ASTKGPSVFPLAPCSRSTSE STAALGCLVK DYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVT VPSSSLGTKT YTCNVDHKPSNTKVDKRVES KYGPPCPPCPAPEFLGGPSV FLFPPKPKDT LMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTK PREEQFNSTY RVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAK GQPREPQVYT LPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENN YKTTTPVLDS DGSFFLYSRLTVDKSRWQEG NVFSCSVMHREALHNHYTQKS LSLSPGKGGG GSGGGGSIDETRAHLLLEK MMRLGGRLVLNTKEELANER LMTLKIAEMK EAMRTLIFPPSMHFFQAKHL IERSQVFNIILRMMPKGAALH	288	CAGGTGCAGCTGGTCCAGAGCGGCGTGGAAGTCAAGAAA CCCGGGGCCTCAGTGAAGGTCAGCTGTAAAGCTTCCGGC TACACCTTCACAACTACTATATGTATTGGGTGAGACAG GCACCAGGACAGGGACTGGAGTGGATGGGCGGGATTAAC CCTAGTAATGGAGGCACTAACTTCAACGAAAAGTTTAAA AACAGGGTGACCCTGACCACAGATTCAAGCACTACCACA GCTTACATGGAGCTGAAGTCCCTGCAGTTTGACGATACA GCCGTGTACTATTGTGCTCGGAGAGACTACAGGTTTCGAT ATGGGCTTTGACTATTGGGGCCAGGGGACTACCGTGACC GTCTCCTCTGCTAGCACCAAGGGCCCATCGGTCTTCCCC CTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCC GCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG GTGACGGTGTGCTGGAACCTCAGGCGCCCTGACCAGCGGC GTGCACACCTTCCCGGCTGTCTACAGTCCTCAGGACTC TACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGC TTGGGCACGAAGACCTACACCTGCAACGTAGATCACAAG CCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAA TATGGTCCCCCATGCCACCATGCCAGCACCTGAGTTC CTGGGGGGACCATCAGTCTTCCTGTTCCCCCAAACCC AAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACG TGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTC CAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAAT GCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACG TACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGAC TGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC AAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAA GCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTG CCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGC CTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC TACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCC TTCTTCCTCTACAGCAGGCTCACCGTGGACAAGAGCAGG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		LHDIGIVTMD WLVRNVTYRPHCHICFTPRG IMQFRFAHPTPRPSEKCSKW ILLEDYRKRV QNVTEFDDSLLRNFTLVTQH PEVIYTNQNVVWSKFETIFF TISGLIHYAP VFRDYVFRSMQEFYEDNVLY MEIRAQLLPVYELSGEHHDE EWSVKTYQEV AQKFVETHPEFIGIKIIYND HRSKDVAVIAESIRMAMGLR IKFPTVVAGF DLVGHEDTGHSLHDYKEALM IPAKDGVKLPYFFHAGETDW QGTSIDRNIL DALMLNTRIGHGFALSKHP AVRTYSWDKDIPIEVCPISN QVLKLVSDLR NHPVATLMATGHPMVISSDD PAMFGAKGLSYDFYEVFMGI GGMKADLRTL KQLAMNSIKYSTLLESEKNT FMEIWKKRWDKFIADVATK		TGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCAT GAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCC CTGTCTCCGGGTAAAGGTGGAGGTGGTTCTGGAGGTGGA GGTAGTATCGACGAAACCAGAGCACACTTACTGCTGAAA GAGAAAATGATGCGCCTGGGCGGGAGATTGGTGTTAAAT ACTAAGGAAGAGCTGGCAAATGAAAGACTCATGACACTG AAGATTGCTGAAATGAAGGAGGCGATGAGGACGCTGATC TTTCCGCCTTCCATGCACTTCTTCCAAGCTAAACACCTG ATCGAAAGATCCCAAGTGTTTAACATCCTGAGGATGATG CCTAAGGGGGCCGCTCTGCACCTTCACGATATTGGGATT GTAACAATGGACTGGCTGGTAAGGAACGTGACATACAGA CCTCATTGCCATATTTGTTTTACTCCCCGAGGAATCATG CAATTCAGGTTTGCCCAACCACTCCTCGGCCAAGCGAG AAGTG TAGTAAGTGGATTTTGCTGGAAGATTACCGTAAG CGCGTG CAGAATGTGACAGAGTTTGATGACTCCCTGCTC CGCAATTTTACCCTGGTGACCCAGCACCCCGAAGTTATA TACACTAACCAAAATGTCGTGTGGTCCAAGTTTGAGACG ATCTTCTTCACGATTT CAGGCTTGATCCACTACGCCCCG GTCTTTCGGGATTATGTGTTTAGGAGTATGCAGGAGTTT TATGAGGATAATGTTCTGTACATGGAGATCCGAGCCCAG CTGCTTCCAGTCTACGAACTATCCGGCGAACACCATGAC GAGGAATGGAGCGTCAAGACCTATCAAGAGGTGGCCCAG AAGTTCGTAGAAACGCATCCAGAGTTCATCGGTATTAAG ATTATCTACAATGATCACCGCTCAAAGGATGTGGCTGTC ATCGCCGAGTCTATACGGATGGCCATGGGCCTGCGGATT AAGTTCCTACCGTCGTCGCCGATTGACCTCGTTGGG CATGAGGATACTGGCCATAGTCTCCATGACTATAAAGAA GCCCTTATGATCCCAGCAAAGGACGGAGTGAAGCTGCC TACTTCTTCCACGCAGGGGAGACCGACTGGCAGGGAAACG AGCATCGACCGGAACATACTTGATGCACTCATGCTTAAT ACCACACGAATCGGCCACGGCTTCGCTCTCTCCAAGCAC CCAGCCGTGAGAACCTACAGCTGGGATAAGGATATCCCC ATCGAGGTTTGTCCCATCAGCAATCAGGTGCTGAAATTG GTGAGTGACCTGAGAAACCACCCAGTCGCAACATTAATG GCCACTGGCCACCCTATGGTGATTTCAAGCGATGATCCA GCCATGTTTCGGAGCAAAGGACTCAGTTACGACTTCTAT GAGGTATTCATGGGTATTGGTGGTATGAAGGCAGACCTG CGGACTCTTAAGCAGTTGGCAATGAACTCAATTAAGTAC TCTACCTTATTGGAGTCTGAAAAGAACACATTTATGGAG ATCTGGAAAAAGCGCTGGGACAAATTCATCGCAGATGTT GCCACAAAA
Human ADA2	284	SIDETRAHLLLKEKMM RLGGRLVLNTKEELAN ERLMTLKIAEMKEAMR TLIFPPSMHFFQAKHLIE RSQVFNILRMMPKGAA LHLHDIGIVTMDWLVR NVTYRPHCHICFTPRGI MQFRFAHPTPRPSEKCS KWILLEDYRKRVQNV TEFDDSLLRNFTLVTQHP EVIYTNQNVVWSKFETI FFTISGLIHYAPVFRDY VFRSMQEFYEDNVLYM EIRARLLPVYELSGEHH DEEWSVKTYQEVAQKF VETHPEFIGIKIISDHR		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		SKDVAVIAESIRMAMG LRIKFPTVVAGFDLVGH EDTGHSLHDYKEALMI PAKDGVKLPYFFHAGE TDWQGTSIDRNILDAL MLNTTRIGHGFALSKHP AVRTYSWKKDPIEVCP ISNQVLKLVSDLRNHPV ATLMATGHPMVISSDD PAMFGAKGLSYDFYEV FMGIGGMKADLRTLKQ LAMNSIKYSTLLESEKN TFMEIWKKRWDKFIAD VATK		

Table 15. Exemplary anti-PD1 VH/VL Pairs

Table 15 provides exemplary anti-PD1 VH/VL pairs for use in the fusion proteins described herein

Exemplary anti-PD1 VL	Exemplary anti-PD1 VH
Anti-PD1 VL5 (SEQ ID NO:12)	Anti-PD1 VH6 (SEQ ID NO: 6)
Anti-PD1 VL6 (SEQ ID NO: 13	Anti-PD1 VH7 (SEQ ID NO: 7)
Anti-PD1 VL1 (SEQ ID NO: 8)	Anti-PD1 VH5 (SEQ ID NO: 5)
Anti-PD1 nVL1 (SEQ ID NO: 8)	Anti-PD1 nVH3 (SEQ ID NO: 149)
Anti-PD1 nVL1 (SEQ ID NO: 8)	Anti-PD1 nVH7 (SEQ ID NO: 157)
Anti PD1 nVL1 (SEQ ID NO: 8)	Anti-PD1 nVH8 (SEQ ID NO: 158)

Table 16 Exemplary anti-PD1-TGFbRII ECD Fusion Protein Sequences

Table 16 provides exemplary anti-PD1-TGFbRII ECD fusion protein sequences. The anti-PD1 fusion proteins comprise a sequence encoding an anti-PD1 variable region of light chain and a sequence encoding an anti-PD1 variable region of heavy chain. ECD stands for extracellular domain. “wt” refers to wild type sequence and “mut” refers to mutant sequence.

Exemplary VL	Exemplary VH-IgG4-ECD
VL5 (SEQ ID NO:15)	VH6-IgG4(wt)-linker-ECD (SEQ NO: 16)
VL5 (SEQ ID NO: 15)	VH6-IgG4(mut)-linker-ECD (SEQ NO: 143)
VL5 (SEQ ID NO:15)	VH6-IgG4(mut)-linker-ECD (SEQ NO: 294)
VL6 (SEQ ID NO: 296	VH7-IgG4(wt)-linker-ECD (SEQ NO: 145)
VL6 (SEQ ID NO: 296)	VH7-IgG4(mut)-linker-ECD (SEQ NO: 144)

VL6 (SEQ ID NO: 296)	VH7-IgG4(mut)-linker-ECD (SEQ NO: 295)
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Table 17 Exemplary anti-PD1-ADA2 Fusion Protein Sequences

Table 17 provides exemplary anti-PD1-ADA2 fusion protein sequences. The anti-PD1 fusion proteins comprise a sequence encoding an anti-PD1 variable region of light chain and a sequence encoding an anti-PD1 variable region of heavy chain. “wt” refers to wild type sequence and “mut” refers to mutant sequence

Exemplary VL	Exemplary VH-IgG4-ADA2
VL5 (SEQ ID NO:12)	VH6-IgG4(mut)-linker-ADA2 (wt) (SEQ NO. 280)
VL5 (SEQ ID NO:12)	VH6-IgG4(mut)-linker-ADA2 (mut 7) (SEQ NO. 281)
VL6 (SEQ ID NO: 13)	VH7-IgG4(mut)-linker-ADA2 (wt) (SEQ NO. 282)
VL6 (SEQ ID NO: 13)	VH7-IgG4(mut)-linker-ADA2 (mut 7) (SEQ NO. 283)

CLAIMS

What is claimed is:

1. A fusion protein comprising:
(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;
wherein one or more polypeptides of the fusion protein are connected by a linker.
2. The fusion protein of claim 1, wherein the linker comprises the amino acid sequence of any one of SEQ ID NOs: 17–34.
3. The fusion protein of claim 1, wherein 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.
6. The fusion protein of claim 1, wherein the first polypeptide comprises an amino acid sequence that differs from the amino acid sequence of SEQ ID NO: 6 or 7 only by conservative amino acid substitutions.

7. The fusion protein of claim 1, wherein the second polypeptide comprises 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.
9. The fusion protein of claim 1, comprising: a polypeptide having at least 95% sequence identity with SEQ ID NO: 296 and a polypeptide having at least 95% sequence identity with SEQ ID NO: 295.
10. The fusion protein of claim 1, comprising: a polypeptide having at least 95% sequence identity with SEQ ID NO: 15 and a polypeptide having at least 95% sequence identity with SEQ ID NO: 294.
11. The fusion protein of claim 1, comprising: the amino acid sequence of SEQ ID NO: 15; and the amino acid sequence of SEQ ID NO: 294.
12. The fusion protein of claim 1, comprising: the amino acid sequence of SEQ ID NO: 296; and the amino acid sequence of SEQ ID NO: 295.
13. The fusion protein of claim 1, wherein the first polypeptide comprises the amino acid sequence of SEQ ID NO: 6 or 7.
14. The fusion protein of claim 1, wherein the second polypeptide comprises the amino acid sequence of SEQ ID NO: 12 or 13.
15. The fusion protein of claim 1, wherein the fourth polypeptide comprises the amino acid sequence of SEQ ID NO: 14.