

OX40-BINDING POLYPEPTIDES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of US Provisional Application No. 62/718,106, filed August 13, 2018, which is incorporated by reference herein in its entirety for any purpose.

FIELD

[0002] The present invention relates to OX40-binding polypeptides, and methods of using OX40-binding polypeptides to modulate the biological activity of OX40. Such methods include, but are not limited to, methods of treating cancer. In some embodiments, the OX40-binding polypeptides are multivalent OX40-binding polypeptides.

BACKGROUND

[0003] The tumor necrosis factor receptor superfamily (TNFRSF) includes several structurally related cell surface receptors. Activation by multimeric ligands is a common feature of many of these receptors, and such activation has therapeutic utility in numerous pathologies if activated properly. Effective agonism of this receptor family may require higher order clustering than is achieved using traditional bivalent antibodies.

[0004] OX40 (TNFRSF4, CD134) is a member of the TNF receptor superfamily, and is expressed on the surface of T cells 24 to 72 hours following T cell activation. Antigen presenting cells in close proximity to activated T cells present OX40 ligand (OX40L) on their surface, which binds and clusters OX40 on T cells sending a co-stimulatory signal that increases T-cell expansion and enhances effector T-cell differentiation. Activation of OX40 therefore serves to maintain an immune response, e.g., by enhancing survival and function of T cells.

[0005] Therefore, there exists a therapeutic need for more potent agonists of OX40.

SUMMARY

[0006] Provided herein are polypeptides comprising at least one VHH domain that binds OX40, wherein the VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 10, a CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 12. In some examples, the VHH domain is humanized. In some examples, the VHH domain comprises a framework 2 (FR2) comprising the amino acid sequence of SEQ ID NO: 22. In some examples, the VHH domain comprises a FR2 comprising the amino acid sequence of SEQ ID NO: 22 and a FR3

comprising the amino acid sequence of SEQ ID NO: 23. In some examples, the VHH domain comprises the amino acid sequence of SEQ ID NO: 9.

[0006a] In one aspect, the present disclosure refers to a polypeptide comprising at least one VHH domain that binds OX40, wherein the VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 10, a CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 12.

[0007] In some examples, the polypeptide comprises two VHH domains. In some examples, the polypeptide comprises three VHH domains. In some examples, the polypeptide comprises four VHH domains. In some examples, the polypeptide comprises at least one binding domain that binds a second antigen other than OX40. In some such examples, the second antigen is selected from PD-1, PD-L1, and 41BB. In some examples, the at least one binding domain that binds a second antigen is an antagonist or an agonist. In some examples, the at least one binding domain that binds a second antigen is a VHH domain.

[0008] In some examples, each VHH domain binds OX40. In some examples, each VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 10, a CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 12. In some examples, each VHH domain comprises a framework 2 (FR2) comprising the amino acid sequence of SEQ ID NO: 22. In some examples, each VHH domain comprises a FR2 comprising the amino acid sequence of SEQ ID NO: 22 and a FR3 comprising the amino acid sequence of SEQ ID NO: 23. In some examples, each VHH domain comprises the amino acid sequence of SEQ ID NO: 9.

[0009] In some examples, the polypeptide comprises an Fc domain. In some examples, the Fc domain comprises an amino acid sequence selected from SEQ ID NOs: 25 and 26. In some examples, the polypeptide comprises the amino acid sequence of SEQ ID NO: 14. In some examples, the polypeptide comprises the amino acid sequence of SEQ ID NO: 15. In another aspect, the present disclosure refers to a polypeptide that binds OX40 comprising the amino acid sequence of SEQ ID NO: 15. In another aspect, the present disclosure refers to a polypeptide that binds OX40 consisting of the amino acid sequence of SEQ ID NO: 15.

[0010] In various examples, the polypeptide provided herein forms a dimer under physiological conditions. In some such examples, the polypeptide comprises an Fc domain.

[0011] In some examples, a polypeptide provided herein increases CD4⁺ and/or CD8⁺ T cell proliferation *in vitro* and/or *in vivo*. In some examples, the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation in the presence of Treg cells. In some examples, the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation *in vitro* by at least 1.5-fold or by at least 2-fold. In some examples, the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation *in vivo* by at least 1.5-fold or by at least 2-fold.

[0012] In some examples, the polypeptide increases CD25 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*. In some examples, the polypeptide increases CD25

expression on CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold or by at least 2-fold. In some examples, the polypeptide increases CD25 expression on CD4⁺ and/or CD8⁺ T cells *in vivo* by at least 1.5-fold or by at least 2-fold.

[0013] In some examples, the polypeptide increases CD71 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*. In some examples, the polypeptide increases CD71 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold or by at least 2-fold. In some examples, the polypeptide increases CD71 expression on CD4⁺ and/or CD8⁺ T cells *in vivo* by at least 1.5-fold or by at least 2-fold.

[0014] In some examples, the polypeptide increases NFκB signaling in CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*. In some examples, the polypeptide increases NFκB signaling in CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold. In some examples, the polypeptide increases NFκB signaling in CD4⁺ and/or CD8⁺ T cells *in vivo* by at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.

[0015] In some examples, the polypeptide increases IFNγ expression in CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*. In some examples, the polypeptide increases IFNγ expression in CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold. In some examples, the polypeptide increases IFNγ expression in CD4⁺ and/or CD8⁺ T cells *in vivo* by at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.

[0016] In various examples, the polypeptide increases the expression of CD25, CD71, and/or IFNγ, and/or increases NFκB signaling in the presence of Treg cells. In various examples, the increase is determined as an average of results from T cells of at least five or at least ten different healthy human donors.

[0017] In various examples, the polypeptide comprising at least one VHH domain that binds OX40 provided herein is an agonist of OX40 biological activity. In some examples, the OX40 is human OX40. In some examples, the polypeptide binds human OX40 with an affinity (K_D) of less than 10 nM, less than 5 nM, less than 2 nM, or less than 1 nM. In some examples, the polypeptide binds cynomolgus monkey OX40 with an affinity (K_D) of less than 10 nM, less than 5 nM, less than 2 nM, or less than 1 nM.

[0018] In some examples, pharmaceutical compositions are provided, comprising a polypeptide comprising at least one VHH domain that binds OX40 provided herein and a pharmaceutically acceptable carrier.

[0018a] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the polypeptide as disclosed herein and a pharmaceutically acceptable carrier.

[0019] In some examples, an isolated nucleic acid is provided that encodes a polypeptide comprising at least one VHH domain that binds OX40 provided herein. In some examples,

a vector is provided that comprises the nucleic acid. In some examples, a host cell comprising the nucleic acid or vector is provided. In some examples, a host cell is provided that expresses a polypeptide comprising at least one VHH domain that binds OX40 provided herein. In some examples, the host cell secretes the OX40-binding polypeptide. In some examples, the host cell is a primary human cell. In some examples, the host cell is a T cell. In some examples, the host cell is a chimeric antigen receptor (CAR)-T cell.

[0019a] In another aspect, the present disclosure refers to an isolated nucleic acid that encodes the polypeptide as disclosed herein. In another aspect, the present disclosure refers to a vector comprising the nucleic acid as disclosed herein. In another aspect, the present disclosure refers to a host cell comprising the nucleic acid as disclosed herein or the vector as disclosed herein. In another aspect, the present disclosure refers to a host cell that expresses the polypeptide as disclosed herein.

[0020] In some examples, a method of producing the polypeptide comprising at least one VHH domain that binds OX40 is provided, comprising incubating the host cell under conditions suitable for expression of the polypeptide. In some examples, the method further comprises isolating the polypeptide.

[0020a] In another aspect, the present disclosure refers to a method of producing the polypeptide as disclosed herein comprising incubating the host cell as disclosed herein under conditions suitable for expression of the polypeptide.

[0021] In some examples, a method of increasing CD4⁺ and/or CD8⁺ T cell proliferation is provided, comprising contacting T cells with a polypeptide comprising at least one VHH domain that binds OX40. In some examples, a method of increasing CD25 expression on CD4⁺ and/or CD8⁺ T cells is provided, comprising contacting T cells with a polypeptide comprising at least one VHH domain that binds OX40. In some examples, a method of increasing CD71 expression on CD4⁺ and/or CD8⁺ T cells is provided, comprising contacting T cells with a polypeptide comprising at least one VHH domain that binds OX40. In some examples, a method of increasing IFN γ expression in CD4⁺ and/or CD8⁺ T cells is provided, comprising contacting T cells with a polypeptide comprising at least one VHH domain that binds OX40. In some examples, a method of increasing NF κ B signaling in CD4⁺ and/or CD8⁺ T cells is provided, comprising contacting T cells with a polypeptide comprising at least one VHH domain that binds OX40. In various examples, the CD4⁺ and/or CD8⁺ T cells are *in vitro*. In various examples, the CD4⁺ and/or CD8⁺ T cells are *in vivo*. In various examples, the CD4⁺ and/or CD8⁺ T cells are in the presence of Treg cells. In various examples, the increase is at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.

[0021a] In another aspect, the present disclosure refers to an *in vitro* method of increasing CD4⁺ and/or CD8⁺ T cell proliferation comprising contacting T cells with the polypeptide as disclosed herein. In another aspect, the present disclosure refers to an *in vitro* method of increasing CD25 expression on CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide as disclosed herein. In another aspect, the present disclosure refers to an *in vitro* method of increasing CD71 expression on CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide as disclosed herein. In another aspect, the present disclosure refers to an *in vitro* method of increasing NFκB signaling in CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide as disclosed herein. In another aspect, the present disclosure refers to an *in vitro* method of increasing IFNγ expression in CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide as disclosed herein.

[0021b] In another aspect, the present disclosure refers to use of the polypeptide as disclosed herein in the manufacture of a medicament for increasing CD4⁺ and/or CD8⁺ T cell proliferation. In another aspect, the present disclosure refers to use of the polypeptide as disclosed herein in the manufacture of a medicament for increasing CD25 expression on CD4⁺ and/or CD8⁺ T cells. In another aspect, the present disclosure refers to use of the polypeptide as disclosed herein in the manufacture of a medicament for increasing CD71 expression on CD4⁺ and/or CD8⁺ T cells. In another aspect, the present disclosure refers to use of the polypeptide as disclosed herein in the manufacture of a medicament for increasing NFκB signaling in CD4⁺ and/or CD8⁺ T cells. In another aspect, the present disclosure refers to use of the polypeptide as disclosed herein in the manufacture of a medicament for increasing IFNγ expression in CD4⁺ and/or CD8⁺ T cells.

[0022] In some examples, methods of treating cancer are provided, comprising administering to a subject with cancer a pharmaceutically effective amount of a polypeptide comprising at least one VHH domain that binds OX40 provided herein. In some examples, methods of treating cancer are provided, comprising administering to a subject with cancer a pharmaceutically effective amount of a host cell that expresses the polypeptide comprising at least one VHH domain that binds OX40 provided herein. In some examples, the host cell secretes the OX40-binding polypeptide. In some examples, the host cell expresses the OX40-binding polypeptide on the surface. In some examples, the cancer is selected from basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon

and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer; gastrointestinal cancer; glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; liver cancer; lung cancer; small-cell lung cancer; non-small cell lung cancer; adenocarcinoma of the lung; squamous carcinoma of the lung; melanoma; myeloma; neuroblastoma; oral cavity cancer; ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma; Hodgkin's lymphoma; non-Hodgkin's lymphoma; B-cell lymphoma; low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; Waldenstrom's macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; and chronic myeloblastic leukemia.

[0022a] In another aspect, the present disclosure refers to use of the polypeptide as disclosed herein, the pharmaceutical composition as disclosed herein, or the host cell as disclosed herein in the manufacture of a medicament for treating cancer.

[0023] In some examples, the method of treating cancer further comprises administering an additional therapeutic agent. In some examples, the additional therapeutic agent is an anti-cancer agent. In some examples, the anti-cancer agent is selected from a chemotherapeutic agent, an anti-cancer biologic, radiation therapy, CAR-T therapy, and an oncolytic virus. In some examples, the additional therapeutic agent is an anti-cancer biologic. In some examples, the anti-cancer biologic is an agent that inhibits PD-1 and/or PD-L1. In some examples, the anti-cancer biologic is selected from nivolumab, pidilizumab, pembrolizumab, durvalumab, atezolizumab, avelumab, AMP-224, BMS-936559, AMP-514, MDX-1105, TSR-042, STI-A1010, and STI-A1110. In some examples, the anti-cancer biologic is an agent that inhibits VISTA, gpNMB, B7H3, B7H4, HHLA2, CD73, CTLA4, or TIGIT. In some examples, the anti-cancer biologic is an antibody. In some examples, the anti-cancer biologic is a cytokine. In some examples, the anti-cancer agent is CAR-T therapy. In some examples, the anti-cancer agent is an oncolytic virus. In some examples, a method of treating cancer provided herein further comprises tumor resection and/or radiation therapy.

BRIEF DESCRIPTION OF THE FIGURES

[0024] FIG. 1 shows nonspecific binding of 1D10v1-Fc and 1D10v6-Fc to untransfected HEK293 cells.

What is claimed is:

1. A polypeptide comprising at least one VHH domain that binds OX40, wherein the VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 10, a CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 12.
2. The polypeptide of claim 1, wherein the VHH domain is humanized.
3. The polypeptide of claim 1 or claim 2, wherein the VHH domain comprises a framework 2 (FR2) comprising the amino acid sequence of SEQ ID NO: 22.
4. The polypeptide of any one of claims 1 to 3, wherein the VHH domain comprises a FR2 comprising the amino acid sequence of SEQ ID NO: 22 and a FR3 comprising the amino acid sequence of SEQ ID NO: 23.
5. The polypeptide of any one of claims 1 to 4, wherein the VHH domain comprises the amino acid sequence of SEQ ID NO: 9.
6. The polypeptide of any one of claims 1 to 5, comprising two VHH domains.
7. The polypeptide of any one of claims 1 to 5, comprising three VHH domains.
8. The polypeptide of any one of claims 1 to 5, comprising four VHH domains.
9. The polypeptide of any one of claims 1 to 8, wherein the polypeptide comprises at least one binding domain that binds a second antigen other than OX40.
10. The polypeptide of claim 9, wherein the second antigen is selected from PD-1, PD-L1, and 41BB.
11. The polypeptide of claim 10, wherein the at least one binding domain that binds a second antigen is an antagonist or an agonist.
12. The polypeptide of any one of claims 5 to 8, wherein each VHH domain binds OX40.
13. The polypeptide of claim 12, wherein each VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 10, a CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 12.
14. The polypeptide of claim 13, wherein each VHH domain comprises a framework 2 (FR2) comprising the amino acid sequence of SEQ ID NO: 22.
15. The polypeptide of claim 13 or claim 14, wherein each VHH domain comprises a FR2 comprising the amino acid sequence of SEQ ID NO: 22 and a FR3 comprising the amino acid sequence of SEQ ID NO: 23.
16. The polypeptide of any one of claims 13 to 15, wherein each VHH domain comprises the amino acid sequence of SEQ ID NO: 9.

17. The polypeptide of any one of claims 1 to 16, wherein the polypeptide comprises an Fc domain.

18. The polypeptide of claim 17, wherein the Fc domain comprises an amino acid sequence selected from SEQ ID NOs: 25 and 26.

19. The polypeptide of any one of claims 1 to 7, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 14.

20. The polypeptide of any one of claims 1 to 7 and 17, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 15.

21. A polypeptide that binds OX40 comprising the amino acid sequence of SEQ ID NO: 15.

22. A polypeptide that binds OX40 consisting of the amino acid sequence of SEQ ID NO: 15.

23. The polypeptide of any one of claims 1 to 22, which forms a dimer under physiological conditions.

24. The polypeptide of any one of claims 1 to 23, wherein the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation *in vitro* and/or *in vivo*.

25. The polypeptide of claim 24, wherein the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation in the presence of Treg cells.

26. The polypeptide of claim 24 or claim 25, wherein the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation *in vitro* by at least 1.5-fold or by at least 2-fold.

27. The polypeptide of any one of claims 1 to 26, wherein the polypeptide increases CD25 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*.

28. The polypeptide of claim 27, wherein the polypeptide increases CD25 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold or by at least 2-fold.

29. The polypeptide of any one of claims 1 to 28, wherein the polypeptide increases CD71 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*.

30. The polypeptide of claim 29, wherein the polypeptide increases CD71 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold or by at least 2-fold.

31. The polypeptide of any one of claims 1 to 30 wherein the polypeptide increases NFκB signaling in CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*.

32. The polypeptide of claim 31, wherein the polypeptide increases NFκB signaling in CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.

33. The polypeptide of any one of claims 1 to 32, wherein the polypeptide increases IFNγ expression in CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*.

34. The polypeptide of claim 33, wherein the polypeptide increases IFN γ expression in CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.

35. The polypeptide of any one of claims 26 to 34, wherein the polypeptide increases the expression in the presence of Treg cells.

36. The polypeptide of any one of claims 24 to 35, wherein the increase is determined as an average of results from T cells of at least five or at least ten different healthy human donors.

37. The polypeptide of any one of claims 1 to 36, which is an agonist of OX40 biological activity.

38. The polypeptide of any one of claims 1 to 37, wherein the OX40 is human OX40.

39. The polypeptide of any one of claims 1 to 38, wherein the polypeptide binds human OX40 with an affinity (K_D) of less than 10 nM, less than 5 nM, less than 2 nM, or less than 1 nM.

40. The polypeptide of any one of claims 1 to 39, wherein the polypeptide binds cynomolgus monkey OX40 with an affinity (K_D) of less than 10 nM, less than 5 nM, less than 2 nM, or less than 1 nM.

41. A pharmaceutical composition comprising the polypeptide of any one of claims 1 to 40 and a pharmaceutically acceptable carrier.

42. An isolated nucleic acid that encodes the polypeptide of any one of claims 1 to 42.

43. A vector comprising the nucleic acid of claim 42.

44. A host cell comprising the nucleic acid of claim 42 or the vector of claim 43.

45. A host cell that expresses the polypeptide of any one of claims 1 to 40.

46. The host cell of claim 44 or claim 45, which secretes the polypeptide of any one of claims 1 to 40.

47. The host cell of any one of claims 44 to 46, which is a primary human cell.

48. The host cell of claim 47, which is a primary human T cell.

49. The host cell of any one of claims 44 to 48, which is a chimeric antigen receptor (CAR)-T cell.

50. A method of producing the polypeptide of any one of claims 1 to 40 comprising incubating the host cell of any one of claims 44 to 46 under conditions suitable for expression of the polypeptide.

51. The method of claim 50, further comprising isolating the polypeptide.

52. An *in vitro* method of increasing CD4⁺ and/or CD8⁺ T cell proliferation comprising contacting T cells with the polypeptide of any one of claims 1 to 40.
53. An *in vitro* method of increasing CD25 expression on CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide of any one of claims 1 to 40.
54. An *in vitro* method of increasing CD71 expression on CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide of any one of claims 1 to 40.
55. An *in vitro* method of increasing NFκB signaling in CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide of anyone of claims 1 to 40.
56. An *in vitro* method of increasing IFNγ expression in CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide of anyone of claims 1 to 40.
57. The method of any one of claims 52 to 56, wherein the CD4⁺ and/or CD8⁺ T cells are in the presence of Treg cells.
58. The method of any one of claims 52 to 57, wherein the increase is at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.
59. Use of the polypeptide of any one of claims 1 to 40, the pharmaceutical composition of claim 41, or the host cell of any one of claims 47 to 49 in the manufacture of a medicament for treating cancer.
60. The use of claim 59, wherein the cancer is selected from basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer; gastrointestinal cancer; glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; liver cancer; lung cancer; small-cell lung cancer; non-small cell lung cancer; adenocarcinoma of the lung; squamous carcinoma of the lung; melanoma; myeloma; neuroblastoma; oral cavity cancer; ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma; Hodgkin's lymphoma; non-Hodgkin's lymphoma; B-cell lymphoma; low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; Waldenstrom's macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; and chronic myeloblastic leukemia.

61. The use of claim 59 or 60, wherein an additional therapeutic agent is to be administered.
62. The use of claim 61, wherein the additional therapeutic agent is an anti-cancer agent.
63. The use of claim 62, wherein the anti-cancer agent is selected from a chemotherapeutic agent, an anti-cancer biologic, radiation therapy, CAR-T therapy, and an oncolytic virus.
64. The use of any one of claims 61 to 63, wherein the additional therapeutic agent is an anti-cancer biologic.
65. The use of claim 64, wherein the anti-cancer biologic is an agent that inhibits PD-1 and/or PD-L1.
66. The use of claim 65, wherein the anti-cancer biologic is selected from nivolumab, pidilizumab, pembrolizumab, durvalumab, atezolizumab, avelumab, AMP-224, BMS-936559, AMP-514, MDX-1105, TSR-042, STI-A1010, and STI-A1110.
67. The use of claim 64, wherein the anti-cancer biologic is an agent that inhibits VISTA, gpNMB, B7H3, B7H4, HHLA2, CD73, CTLA4, or TIGIT.
68. The use of any one of claims 64 to 67, wherein the anti-cancer agent is an antibody.
69. The use of claim 64, wherein the anti-cancer biologic is a cytokine.
70. The use of claim 64, wherein the anti-cancer agent is CAR-T therapy.
71. The use of claim 64, wherein the anti-cancer agent is an oncolytic virus.
72. The use of any one of claims 59 to 71, further comprising tumor resection and/or radiation therapy.
73. Use of the polypeptide of any one of claims 1 to 40 in the manufacture of a medicament for increasing CD4⁺ and/or CD8⁺ T cell proliferation.
74. Use of the polypeptide of any one of claims 1 to 40 in the manufacture of a medicament for increasing CD25 expression on CD4⁺ and/or CD8⁺ T cells.
75. Use of the polypeptide of any one of claims 1 to 40 in the manufacture of a medicament for increasing CD71 expression on CD4⁺ and/or CD8⁺ T cells.
76. Use of the polypeptide of any one of claims 1 to 40 in the manufacture of a medicament for increasing NFκB signaling in CD4⁺ and/or CD8⁺ T cells.
77. Use of the polypeptide of any one of claims 1 to 40 in the manufacture of a medicament for increasing IFNγ expression in CD4⁺ and/or CD8⁺ T cells.
78. The use of any one of claims 73 to 77, wherein the CD4⁺ and/or CD8⁺ T cells are in the presence of Treg cells.

79. The use of any one of claims 73 to 78, wherein the increase is at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.