

WHAT IS CLAIMED:

1. A bispecific antibody comprising
 - (a) a first polypeptide from N-terminus to C-terminus comprising (i) a first single chain variable fragment (scFv) that binds to a tumor-associated antigen (TAA), (ii) an immunoglobulin constant region, and (iii) an scFv that binds to CD3; and
 - (b) a second polypeptide from N-terminus to C-terminus comprising (i) a second scFv that binds to the TAA, and (ii) an immunoglobulin constant region,wherein the bispecific antibody does not contain a second CD3-binding domain.
2. The bispecific antibody of claim 1, wherein the TAA is PSMA, HER2, or BCMA.
3. The bispecific antibody of claim 1, wherein the TAA is PSMA.
4. The bispecific antibody of any one of claims 1-3, wherein the first polypeptide and the second polypeptide are joined by at least one disulfide bond.
5. The bispecific antibody of any one of claims 1-4, wherein the first scFv that binds to the TAA is in the VH-VL orientation or the VL-VH orientation.
6. The bispecific antibody of any one of claims 1-5, wherein the second scFv that binds to the TAA is in the VH-VL orientation or the VL-VH orientation.
7. The bispecific antibody of any one of claims 1-6, wherein the first scFv that binds to the TAA and the second scFv that binds to the TAA are the same.
8. The bispecific antibody of any one of claims 1-7, wherein the scFv that binds to CD3 is in the VH-VL orientation or the VL-VH orientation.
9. The bispecific antibody of any one of claims 1-8,
wherein the immunoglobulin constant region in the first polypeptide comprises a knob mutation and/or the immunoglobulin constant region in the second polypeptide comprises a hole mutation; or
wherein the immunoglobulin constant region in the first polypeptide comprises a hole mutation and/or the immunoglobulin constant region in the second polypeptide comprises a knob mutation.
10. The bispecific antibody of claim 9, wherein immunoglobulin constant region comprising a knob mutation comprises the amino acid sequence of SEQ ID NO:66 and/or the immunoglobulin constant region comprising a hole mutation comprises the amino acid sequence of SEQ ID NO: 68.

11. The bispecific antibody of any one of claims 1-10, wherein the immunoglobulin constant region comprises one, two, three, four, five or more amino acid substitutions and/or deletions compared to a wild-type immunoglobulin constant region to (i) prevent binding of FcγR1 and/or FcγRIIIb; or (ii) prevent or reduce CDC activity.
12. The bispecific antibody of any one of claims 1-11, wherein the immunoglobulin constant region comprises a IgG1 CH2 domain comprising the substitutions E233P, L234A, L235A, G237A, and K322A and a deletion of G236 according to the EU numbering system.
13. The bispecific antibody of any one of claims 1-12, wherein the immunoglobulin constant region comprises an immunoglobulin CH2 and CH3 domains of IgG1.
14. The bispecific antibody of any one of claims 1-13, wherein the bispecific antibody does not contain a CHI domain.
15. The bispecific antibody of any one of claims 1-14, wherein the first scFv that binds to the TAA, the scFv that binds to CD3, and/or the second scFv that binds to the TAA comprises (i) a glycine-serine linker; or (ii) a glycine-serine linker comprising the amino acid sequence (Gly₄Ser)_n, wherein n=1-5, and optionally wherein n=4.
16. The bispecific antibody of any one of claims 1-15, wherein the first polypeptide and/or the second polypeptide further comprises at least one linker between an scFv and an immunoglobulin constant domain, optionally wherein the linker comprises a hinge region, optionally wherein the hinge is an IgG1 hinge region, and optionally wherein the hinge comprises the amino acid sequence of SEQ ID NO: 156.
17. The bispecific antibody of any one of claims 3-16, wherein the first scFv that binds to PSMA and/or the second scFv that binds to PSMA is capable of binding to cynomolgus PSMA, and optionally wherein the first scFv that binds to PSMA and/or the second scFv that binds to cynomolgus PSMA has an EC₅₀ of no more than 5-times greater than the EC₅₀ for binding to human PSMA.
18. The bispecific antibody of any one of claims 1-17, wherein the bispecific antibody is capable of binding to the TAA and CD3 simultaneously.
19. The bispecific antibody of claim 1-18, wherein the scFv that binds to CD3 binds to CD3ε.
20. The bispecific antibody of any one of claims 3-19, wherein the first scFv that binds to PSMA comprises a variable heavy (VH) complementarity-determining region (CDR)1, VH CDR2, and VH CDR3 comprising the amino acid sequences of SEQ ID NOs: 70, 72, and 74,

- respectively, and comprises a variable light (VL) CDR1, VL CDR2, and VL CDR3 comprising the amino acid sequences of SEQ ID NOs: 76, 78, and 80, respectively.
21. The bispecific antibody of any one of claims 3-20, wherein the first scFv that binds to PSMA comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 82 and comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 84.
 22. The bispecific antibody of any one of claims 3-21, wherein the first scFv that binds to PSMA comprises the amino acid sequence of SEQ ID NO: 86.
 23. The bispecific antibody of any one of claims 1-22, wherein the scFv that binds to CD3 comprises a VH CDR1, VH CDR2, and VH CDR3 comprising the amino acid sequences of SEQ ID NOs: 88, 90, and 92, respectively, and comprises a VL CDR1, VL CDR2, and VL CDR3 comprising the amino acid sequences of SEQ ID NOs: 94, 96, and 98, respectively.
 24. The bispecific antibody of any one of claims 1-23, wherein the scFv that binds to CD3 comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 100 and comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 102.
 25. The bispecific antibody of any one of claims 1-24, wherein the scFv that binds to CD3 comprises the amino acid sequence of SEQ ID NO: 104 or 110.
 26. The bispecific antibody of any one of claims 3-25, wherein the second scFv that binds to PSMA comprises a VH CDR1, VH CDR2, and VH CDR3 comprising the amino acid sequences of SEQ ID NOs: 70, 72, and 74, respectively, and comprises a VL CDR1, VL CDR2, and VL CDR3 comprising the amino acid sequences of SEQ ID NOs: 76, 78, and 80, respectively.
 27. The bispecific antibody of any one of claims 3-26, wherein the second scFv that binds to PSMA comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 82 and comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 84.
 28. The bispecific antibody of any one of claims 3-27, wherein the second scFv that binds to PSMA comprises the amino acid sequence of SEQ ID NO: 86.
 29. The bispecific antibody of any one of claims 3-28, wherein the first polypeptide comprises the amino acid sequence of SEQ ID NO: 106, 178, or 112, and optionally wherein the second polypeptide comprises the amino acid sequence of SEQ ID NO: 108.
 30. The bispecific antibody of any one of claims 1-29, wherein the antibody is capable of one or more of the following:
 - (i) promoting expansion of CD8⁺ T cells and/or CD4⁺ T cells;

- (ii) activating CD8⁺ T cells and/or CD4⁺T cells;
 - (iii) increasing central memory T cells (TCM) and/or effector memory T cells (TEM);
 - (iv) decreasing naive and/or terminally differentiated T cells (Teff);
 - (v) decreasing secretion of IFN- γ , IL-2, IL-6, TNF- α , Granzyme B, IL-10, and/or GM-CSF;
and
 - (vi) increasing signaling of NF κ B, NFAT, and/or ERK signaling pathways.
31. An antibody or antigen-binding fragment thereof comprising a PSMA-binding domain, wherein the PSMA-binding domain comprises a VH and a VL, wherein the VH comprises the amino acid sequence of SEQ ID NO:82.
 32. An antibody or antigen-binding fragment thereof comprising a PSMA-binding domain, wherein the PSMA-binding domain comprises a VH and a VL, wherein the VL comprises the amino acid sequence of SEQ ID NO:84.
 33. The antibody or antigen-binding fragment thereof of claim 31 or 32, wherein the VH comprises the amino acid sequence of SEQ ID NO:82, and the VL comprises the amino acid sequence of SEQ ID NO:84.
 34. An antibody or antigen-binding fragment thereof comprising a CD3 antigen-binding domain, wherein the CD3 antigen-binding domain comprises a VH and a VL, wherein the VH comprises the amino acid sequence of SEQ ID NO: 100.
 35. An antibody or antigen-binding fragment thereof comprising a CD3 antigen-binding domain, wherein the CD3 antigen-binding domain comprises a VH and a VL, wherein the VL comprises the amino acid sequence of SEQ ID NO: 102.
 36. The antibody or antigen-binding fragment thereof of claim 34 or 35, wherein the VH comprises an amino acid sequence of SEQ ID NO: 100, and the VL comprises an amino acid sequence of SEQ ID NO: 102.
 37. The antibody or antigen-binding fragment thereof of any one of claims 31-36, wherein the antibody is an IgG antibody, optionally wherein the IgG antibody is an IgG1 antibody, optionally wherein the antibody further comprises a heavy chain constant region and a light chain constant region, optionally wherein the heavy chain constant region is a human IgG1 heavy chain constant region, and optionally wherein the light chain constant region is a human IgGk light chain constant region.

38. The antibody or antigen-binding fragment thereof of any one of claims 31-36, wherein the antibody comprises an a Fab, Fab', F(ab')₂, scFv, disulfide linked Fv, or scFv-Fc, and optionally wherein the antibody comprises a scFv.
39. The antibody or antigen-binding fragment thereof of any one of claims 31-33 and 38, wherein the antibody or antigen-binding fragment comprises the amino acid sequence of SEQ ID NO:86.
40. The antibody or antigen-binding fragment thereof of any one of claims 31-36 and 38, wherein the antibody or antigen-binding fragment comprises the amino acid sequence of SEQ ID NO: 104.
41. The antibody or antigen-binding fragment thereof of any one of claims 31-40, wherein the antibody or fragment is bispecific, optionally wherein the bispecific antibody or fragment comprises an antigen-binding domain that specifically binds PSMA and an antigen-binding domain that specifically binds CD3, and optionally wherein (i) the antigen-binding domain that specifically binds PSMA comprises the amino acid sequences of SEQ ID NOs: 82 and 84, and/or (ii) the antigen-binding domain that specifically binds CD3 comprises the amino acid sequence of SEQ ID NOs: 100 and 102.
42. The antibody or antigen-binding fragment thereof of claim 41, wherein the antigen-binding domain that specifically binds PSMA comprises a VH and a VL in the VH-VL orientation or the VL-VH orientation.
43. The antibody or antigen-binding fragment thereof of any one of claims 41-42, wherein the antigen-binding domain that specifically binds CD3 comprises a VH and a VL in the VH-VL orientation or the VL-VH orientation.
44. The antibody or antigen-binding fragment thereof of claim 41, wherein the antigen-binding domain that specifically binds PSMA comprises a scFv that comprises the amino acid sequence of SEQ ID NO:86; or wherein the antigen-binding domain that specifically binds to CD3 comprises a scFv that comprises the amino acid sequence of SEQ ID NO: 104.
45. The antibody or antigen-binding fragment thereof of any one of claims 34-44, wherein the antibody is (i) monovalent for CD3; or (ii) bivalent for CD3.
46. The antibody or antigen-binding fragment thereof of any one of claims 31-45, wherein the antibody is (i) bivalent for PSMA, or (ii) monovalent for PSMA.

47. The antibody or antigen-binding fragment thereof of any one of claims 31-44, wherein the antibody or fragment comprises a polypeptide comprising, in order from amino-terminus to carboxyl-terminus, (i) a first single chain variable fragment (scFv), (ii) a linker, optionally wherein the linker is a hinge region, (iii) an immunoglobulin constant region, and (iv) a second scFv, wherein (a) the first scFv comprises a human CD3 antigen-binding domain, and the second scFv comprises a human PSMA antigen-binding domain or (b) the first scFv comprises a human PSMA antigen-binding domain and the second scFv comprises a human CD3 antigen-binding domain.
48. The antibody or antigen-binding fragment thereof of any one of claims 31-46, wherein the antibody or fragment comprises a knob mutation and a hole mutation.
49. A bispecific antibody comprising:
 - (a) a first polypeptide from N-terminus to C-terminus comprising (i) a first single chain variable fragment (scFv) that binds to PSMA comprising the amino acid sequence of SEQ ID NO:86, (ii) a linker comprising the amino acid sequence of SEQ ID NO: 156, (iii) an immunoglobulin constant region comprising the amino acid sequence of SEQ ID NO:66, and (iv) an scFv that binds to CD3 comprising the amino acid sequence of SEQ ID NO: 104; and
 - (b) a second polypeptide from N-terminus to C-terminus comprising (i) a second scFv that binds to PSMA comprising the amino acid sequence of SEQ ID NO:86, (ii) a linker comprising the amino acid sequence of SEQ ID NO: 156, and (iii) an immunoglobulin constant region comprising the amino acid sequence of SEQ ID NO:68,wherein the bispecific antibody does not contain a second CD3-binding domain.
50. A bispecific antibody that binds to PSMA and CD3, wherein the bispecific antibody comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 106, 178, or 112 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 108, and wherein the bispecific antibody only contains one CD3-binding domain.
51. A bispecific antibody that binds to PSMA and CD3, wherein the bispecific antibody consists of a first polypeptide comprising the amino acid sequence of SEQ ID NO: 106, 178, or 112 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 108.
52. A polynucleotide encoding the bispecific antibody of any one of claims 1-30 and 49-51 or the antibody of any one of claims 31-48.

53. A vector comprising the polynucleotide of claim 52, optionally wherein the vector is an expression vector.
54. A host cell comprising the polynucleotide of claim 52 or the vector of claim 53.
55. A host cell comprising a combination of polynucleotides that encode the bispecific antibody of any one of claims 1-30 and 49-51 or the antibody of any one of claims 31-48.
56. The host cell of claim 55, wherein the polynucleotides are encoded on (i) a single vector; or (ii) multiple vectors.
57. The host cell of any one of claims 55-56, which is selected from the group consisting of a CHO, HEK293, or COS cell.
58. A method of producing a bispecific antibody that specifically binds to human PSMA and human CD3 comprising culturing the host cell of any one of claims 55-57 so that the antibody is produced, optionally further comprising recovering the antibody.
59. A method for detecting PSMA and CD3 in a sample, the method comprising contacting said sample with the bispecific antibody of any one of claims 1-30 and 49-51, optionally wherein the sample comprises cells.
60. A pharmaceutical composition comprising the bispecific antibody of any one of claims 1-30 and 49-51 or the antibody of any one of claims 31-48, and a pharmaceutically acceptable excipient.
61. The bispecific antibody of any one of claims 1-30 and 49-51 or the pharmaceutical composition of claim 60, for use in increasing T cell proliferation, wherein the antibody or pharmaceutical composition is to be contacted with a T cell.
62. The bispecific antibody or pharmaceutical composition of claim 61, wherein the T cell is a CD4⁺ T cell or a CD8⁺ T cell.
63. The bispecific antibody or pharmaceutical composition of claim 61 or 62, wherein the cell is in a subject and the antibody or the pharmaceutical composition is to be administered to the subject.
64. The bispecific antibody of any one of claims 1-30 and 49-51 or the pharmaceutical composition of claim 60, for use in enhancing an immune response in a subject.
65. The bispecific antibody of any one of claims 1-30 and 49-51 or the pharmaceutical composition of claim 60, for use in inducing redirected T-cell cytotoxicity (RTCC) against a cell expressing prostate-specific membrane antigen (PSMA), wherein the antibody or the

pharmaceutical composition is to be contacted with said PSMA-expressing cell under conditions whereby RTCC against the PSMA-expressing cell is induced.

66. Use of the bispecific antibody of any one of claims 1-30 and 49-51 or the pharmaceutical composition of claim 60 in the manufacture of a medicament for treating a disorder characterized by overexpression of prostate-specific membrane antigen (PSMA).
67. The use of claim 66, wherein the bispecific antibody or the pharmaceutical composition induces redirected T-cell cytotoxicity (RTCC) in the subject.
68. The use of claim 66 or 67, wherein the bispecific antibody is configured to
 - (i) promote expansion or proliferation of CD8⁺ and/or CD4⁺ T cells;
 - (ii) activate CD8⁺ and/or CD4⁺ T cells;
 - (iii) increase central memory T cells (TCM) and/or effector memory T cells (TEM);
 - (iv) decrease naive and/or terminally differentiated T cells (Teff);
 - (v) decrease secretion of IFN- γ , IL-2, IL-6, TNF- α , Granzyme B, IL-10, and/or GM-CSF; or
 - (vi) increase signaling of NF κ B, NFAT, and/or ERK signaling pathways.
69. The use of claim 66, wherein the disorder is a cancer, optionally wherein the cancer is selected from the group consisting of prostate cancer, PSMA(+) cancer, metastatic prostate cancer, clear cell renal carcinoma, bladder cancer, lung cancer, colorectal cancer, and gastric cancer, optionally wherein the cancer is prostate cancer, and optionally wherein the prostate cancer is castrate-resistant prostate cancer.
70. The use of claim 66, wherein the disorder is a prostate disorder, optionally wherein the prostate disorder is selected from the group consisting of prostate cancer and benign prostatic hyperplasia.
71. The bispecific antibody of any one of claims 1-30 and 49-51 or the pharmaceutical composition of claim 60, for use in treating a disorder characterized by overexpression of prostate-specific membrane antigen (PSMA).