

CLAIMS

1. A pharmaceutical composition comprising:
 - (a) a bispecific antibody or antigen-binding fragment thereof that specifically binds 4-1BB and OX40;
 - (b) about 5mM to about 15 mM of a stability promoting buffer;
 - (c) about 25 mM to about 50 mM of a stabilizing amino acid;
 - (d) about 2% to about 8% w/v of a sugar; and
 - (e) about 0.01% to about-0.03% of a surfactant.
2. The pharmaceutical composition of claim 1, wherein the stability promoting buffer is selected from the group consisting of succinate, histidine, citrate, and glutamate, optionally wherein the stability promoting buffer is present in the composition in a concentration of about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, or about 15mM, optionally wherein the stability promoting buffer is glutamate, and optionally wherein the glutamate is present in the composition in a concentration of about 5 mM.
3. The pharmaceutical composition of claim 1 or 2, wherein the stabilizing amino acid is selected from the group consisting of arginine, methionine, leucine, and glycine, optionally wherein the stabilizing amino acid is present in the composition in a concentration of about 25 mM, about 26 mM, about 27 mM, about 28 mM, about 29 mM, about 30 mM, about 31 mM, about 32 mM, about 33 mM, about 34 mM, about 35 mM, about 36 mM, about 37 mM, about 38 mM, about 39 mM, about 40 mM, about 41 mM, about 42 mM, about 43 mM, about 44 mM, about 45 mM, about 46 mM, about 47 mM, about 48 mM, about 49 mM, or about 50 mM, optionally wherein the stabilizing amino acid is leucine, and optionally wherein the leucine is present in the composition in a concentration of about 40 mM.
4. The pharmaceutical composition of any one of claims 1-3, wherein the sugar is selected from the group consisting of: sucrose, trehalose, and sorbitol, optionally wherein the

sugar is present in the composition in a concentration from about 2% w/v to about 8% w/v, optionally wherein the sugar is present in the composition in a concentration of about 2% w/v, about 3% w/v, about 4% w/v, about 5% w/v, about 6% w/v, about 7% w/v, or about 8% w/v, optionally wherein the sugar is sucrose, and optionally wherein the sucrose is present in the composition in a concentration of about 3% w/v.

5. The pharmaceutical composition of any one of claims 1-4, further comprising a surfactant, optionally wherein the surfactant is Polysorbate-80, optionally wherein the Polysorbate-80 is present in the composition in a concentration of from about 0.01% w/v to about 0.03% w/v, or wherein the Polysorbate-80 is present in the composition in a concentration of about 3% w/v.
6. The pharmaceutical composition of any one of claims 1-5, wherein the pH of the composition is in a range from about 4.3 to about 4.7, optionally wherein the pH of the composition is about 4.5.
7. The pharmaceutical composition of any one of claims 1-6, wherein the bispecific antibody or antigen-binding fragment thereof is present in the composition in a concentration of about 10 mg/mL to about 50 mg/mL.
8. The pharmaceutical composition of any one of claims 1-7, wherein the pharmaceutical composition does not comprise sodium chloride (NaCl).
9. The pharmaceutical composition of any one of claims 1-8, wherein the 4-1BB x OX40 bispecific antibody or antigen-binding fragment thereof has a purity of about >90%.
10. The pharmaceutical composition of any one of claims 1-9, comprising:
 - (a) the 4-1BB x OX40 bispecific antibody or antigen-binding fragment thereof in a concentration of about 10 mg/mL;
 - (b) about 10 mM glutamate;
 - (c) about 40 mM leucine;

(d) about 3% w/v sucrose; and

(e) about 0.02% polysorbate-80

wherein the pH of the composition is from about 4.3 to about 4.7, optionally wherein the pH of the composition is 4.5.

11. The pharmaceutical composition of any one of claims 1-10, wherein the bispecific antibody or antigen-binding fragment thereof comprises a single chain Fv (scFv).
12. The pharmaceutical composition of any one of claims 1-11, wherein the bispecific antibody or antigen-binding fragment thereof comprises a Fab, Fab', F(ab')₂, scFv, disulfide linked Fv, or scFv-Fc.
13. The pharmaceutical composition of any one of claims 1-12, wherein the bispecific antibody or antigen-binding fragment thereof comprises a human 4-1BB antigen-binding domain and a human OX40 antigen-binding domain, wherein:
 - (a) the 4-1BB antigen-binding domain comprises a (i) a VH-CDR 1 comprising the amino acid sequence of SEQ ID NO:1; (ii) a VH-CDR2 comprising the amino acid sequence of SEQ ID NO:2; (iii) a VH-CDR3 comprising the amino acid sequence of SEQ ID NO:3; (iv) a light chain variable domain (VL)-CDR1 comprising the amino acid sequence of SEQ ID NO:4; (v) a VL-CDR2 comprising the amino acid sequence of SEQ ID NO:5; and (vi) a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:6; and
 - (b) the OX40 antigen-binding domain comprises a (i) a VH-CDR1 comprising the amino acid sequence of SEQ ID NO:7; (ii) a VH-CDR2 comprising the amino acid sequence of SEQ ID NO:8; (iii) a VH-CDR3 comprising the amino acid sequence of SEQ ID NO:9; (iv) a VL-CDR1 comprising the amino acid sequence of SEQ ID NO:10; (v) a VL-CDR2 comprising the amino acid sequence of SEQ ID NO:11; and (vi) a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:12.

14. The pharmaceutical composition of any one of claims 1-13, wherein the bispecific antibody or antigen-binding fragment thereof comprises a human 4-1BB antigen-binding domain and a human OX40 antigen-binding domain, wherein:
 - (a) the 4-1BB antigen-binding domain comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 14 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15; and
 - (b) the OX40 antigen-binding domain comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 17 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 18.
15. The pharmaceutical composition of any one of claims 1-14, wherein the 4-1BB x OX40 bispecific antibody or antigen-binding fragment thereof comprises a human 4-1BB antigen-binding domain and a human OX40 antigen-binding domain, wherein:
 - (a) the 4-1BB antigen-binding domain comprises a scFv comprising the amino acid sequence of SEQ ID NO: 16; and
 - (b) the OX40 antigen-binding domain comprises a scFv comprising the amino acid sequence of SEQ ID NO: 19.
16. The pharmaceutical composition of any one of claims 1-15, wherein the bispecific antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NO: 13.
17. A pharmaceutical composition, comprising:
 - (a) 10 mg/mL of a 4-1BB x OX40 bispecific antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof comprises:
 - (i) a 4-1BB antigen-binding domain comprising a VH-CDR 1 comprising the amino acid sequence of SEQ ID NO:1; a VH-CDR2 comprising the amino acid sequence of SEQ ID NO:2; a VH-CDR3 comprising the amino acid sequence of SEQ ID NO:3; a light chain variable domain (VL)-CDR1 comprising the amino acid sequence of SEQ ID NO:4; a VL-CDR2 comprising the amino acid sequence of SEQ ID NO:5; and a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:6; and

an OX40 antigen-binding domain comprising a VH-CDR1 comprising the amino acid sequence of SEQ ID NO:7; a VH-CDR2 comprising the amino acid sequence of SEQ ID NO:8; a VH-CDR3 comprising the amino acid sequence of SEQ ID NO:9; a VL-CDR1 comprising the amino acid sequence of SEQ ID NO:10; a VL-CDR2 comprising the amino acid sequence of SEQ ID NO:11; and a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:12;

(ii) a 4-1BB antigen-binding domain comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 14 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15; and

a OX40 antigen-binding domain comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 17 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 18;

(iii) a 4-1BB antigen-binding domain comprising a scFv comprising the amino acid sequence of SEQ ID NO: 16; and an OX40 antigen-binding domain comprising a scFv comprising the amino acid sequence of SEQ ID NO: 19; or

(iv) the amino acid sequence of SEQ ID NO: 13;

(b) about 5mM to about 15 mM glutamate;

(c) about 25 mM to about 50 mM leucine;

(d) about 2% to about 8% w/v sucrose; and

(e) about 0.01% to about-0.03% polysorbate-80;

wherein the pH of the composition is from about 4.3 to about 4.7.

18. A pharmaceutical composition, comprising:

(a) 10 mg/mL of a 4-1BB x OX40 bispecific antibody or antigen-binding fragment thereof; wherein the antibody or antigen-binding fragment thereof comprises:

(i) a 4-1BB antigen-binding domain comprising a VH-CDR 1 comprising the amino acid sequence of SEQ ID NO:1; a VH-CDR2 comprising the amino acid sequence of SEQ ID NO:2; a VH-CDR3 comprising the amino acid sequence of SEQ ID NO:3; a light chain variable domain (VL)-CDR1 comprising the amino acid sequence of SEQ ID NO:4; a VL-CDR2 comprising the amino acid sequence of SEQ ID NO:5; and a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:7; and

(ii) an OX40 antigen-binding domain comprising a VH-CDR1 comprising the amino acid sequence of SEQ ID NO:7; a VH-CDR2 comprising the amino acid sequence of SEQ ID NO:8; a VH-CDR3 comprising the amino acid sequence of SEQ ID NO:9; a VL-CDR1 comprising the amino acid sequence of SEQ ID NO:10; a VL-CDR2 comprising the amino acid sequence of SEQ ID NO:11; and a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:12;

(b) 10 mM glutamate;

(c) 40 mM leucine;

(d) 3% w/v sucrose; and

(e) 0.02% polysorbate-80;

wherein the pH of the composition is 4.5.

19. The pharmaceutical composition of claim 18, wherein

(a) the 4-1BB antigen-binding domain comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 14 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15; and

(b) the OX40 antigen-binding domain comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 17 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 18.

20. The pharmaceutical composition of any one of claims 1-19, wherein the pharmaceutical formulation is a liquid.

21. The pharmaceutical composition of any one of claims 13-15 and 17-20, wherein the human 4-1BB binding domain and the human OX40 binding domain are on the same polypeptide.

22. The pharmaceutical composition of claim 21, wherein the human 4-1BB binding domain is N-terminal to the human OX40 binding domain, or wherein the human 4-1BB binding domain is C-terminal to the human OX40 binding domain.

23. The pharmaceutical composition of any one of claims 1-15 and 17-22, wherein the antibody or antigen-binding fragment thereof comprises an immunoglobulin constant region, optionally wherein the immunoglobulin constant region comprises immunoglobulin CH2 and CH3 domains of IgG1.
24. The pharmaceutical composition of any one of claims 1-23, wherein the antibody does not contain a CH1 domain.
25. The pharmaceutical composition of claim 23 or 24, wherein the immunoglobulin constant region comprises a human IgG1 CH2 domain comprising the substitutions E233P, L234A, L235A, G237A, and K322A and a deletion of G236 according to the EU numbering system.
26. The pharmaceutical composition of any one of claims 13-15 and 17-25, wherein the antibody or antigen-binding fragment thereof comprises a linker between an immunoglobulin constant region and the human 4-1BB binding domain and/or between an immunoglobulin constant region and the human OX40 binding domain, optionally wherein the linker between the immunoglobulin constant region and the human 4-1BB binding domain and/or between the immunoglobulin constant region and the human OX40 binding domain comprises 10- 30 amino acids, 15-30 amino acids, or 20-30 amino acids, and optionally wherein the linker between the immunoglobulin constant region and the human 4-1BB binding domain or between the immunoglobulin constant region and the human OX40 binding domain comprises the amino acid sequence (Gly4Ser)_n, wherein n=1-5 (SEQ ID NO:21), optionally wherein n=1.
27. The pharmaceutical composition of any one of claims 13-26, wherein the antibody comprises a dimer of two polypeptides, each polypeptide comprising in order from amino-terminus to carboxyl-terminus, a first scFv, a hinge region, an immunoglobulin constant region, and a second scFv, wherein (a) the first scFv comprises a human 4-1BB antigen-binding domain, and the second scFv comprises a human OX40 antigen-binding domain or (b) the first scFv comprises a human OX40 antigen-binding domain and the

second scFv comprises a human 4-1BB antigen-binding domain, optionally wherein the dimer is a homodimer.

28. The pharmaceutical composition of any one of claims 1-27, wherein the pharmaceutical formulation can be stored at -20 °C.
29. The pharmaceutical composition of any one of claims 1-28, having no significant change in pH after storage at -20 °C, after about 3 weeks, about 6 weeks, about 3 months, about 6 months, and about 7 months.
30. The pharmaceutical composition of any one of claims 1-29, having no significant change in the percent High Molecular Weight content (%HMW) after storage at -20 °C, after about 3 weeks, about 6 weeks, about 3 months, about 6 months, or about 7 months, and/or wherein the change in the %HMW after storage at -20 °C, after about 3 weeks, about 6 weeks, about 3 months, about 6 months, or about 7 months is less than 5%, and/or having no significant change in the %HMW after one, two, or three freeze/thaw cycles, and/or wherein the change in the %HMW after one, two, or three freeze/thaw cycles is less than 5%.
31. The pharmaceutical composition of claim 30, wherein the %HMW is measured by size exclusion ultra pressure liquid chromatography (SE-UPLC).
32. The pharmaceutical composition of any one of claims 1-31, having no significant change in aggregate formation after storage at -20 °C, after about 3 weeks, about 6 weeks, about 3 months, about 6 months, or about 7 months, as measured by size exclusion ultra pressure liquid chromatography (SE-UPLC).
33. Use of the pharmaceutical composition of any one of claims 1-32 in the manufacture of a medicament for treating cancer in a subject.

34. The use of claim 33, wherein the cancer is a solid tumor cancer, and/or wherein the cancer is a sarcoma, a carcinoma, or a lymphoma, and/or wherein the cancer is selected from the group consisting of a melanoma, kidney cancer, pancreatic cancer, lung cancer, stomach cancer, colon / intestinal cancer, prostate cancer, ovarian cancer, breast cancer, liver cancer, brain cancer, or a hematological cancer, and/or wherein the subject is a human, and/or wherein the subject expresses 4-1BB and OX40 on tumor infiltrating lymphocytes.
35. The pharmaceutical composition of any one of claims 1-32 for use in the treatment of cancer.
36. The pharmaceutical composition for use of claim 35, wherein the cancer is a solid tumor cancer, and/or wherein the cancer is a sarcoma, carcinoma, or lymphoma, and/or wherein the cancer is selected from the group consisting of a melanoma, kidney cancer, pancreatic cancer, lung cancer, colon / intestinal cancer, stomach cancer, prostate cancer, ovarian cancer, breast cancer, liver cancer, brain cancer, or a hematological cancer.