

T cells (e.g., proliferation of CD8⁺ T cells), improved ability to reduce and/or eliminate cancer proliferation *in vivo* (e.g., at a lower dose).

[0004] In some examples, an anti-4-1BB antibody or antigen-binding antibody fragment includes 1, 2, or 3 heavy chain CDR sequences that are or include a sequence of SEQ ID NOs: 5 to 8. In some examples, an anti-4-1BB antibody or antigen-binding antibody fragment includes one or more of: a heavy chain CDR1 that is or includes a sequence of SEQ ID NO: 5, a heavy chain CDR2 that is or includes a sequence of SEQ ID NO: 6 and a heavy chain CDR3 that is or includes a sequence of SEQ ID NO: 7 or 8. In some examples, an anti-4-1BB antibody or antigen-binding antibody fragment includes each of: a heavy chain CDR1 that is or includes a sequence of SEQ ID NO: 5, a heavy chain CDR2 that is or includes a sequence of SEQ ID NO: 6 and a heavy chain CDR3 that is or includes a sequence of SEQ ID NO: 7 or 8.

[0005] In some examples, an anti-4-1BB antibody or antigen-binding antibody fragment includes 1, 2, or 3 light chain CDR sequences that are or include a sequence of SEQ ID NOs: 1-4. In some examples, an anti-4-1BB antibody or antigen-binding antibody fragment includes one or more of: a light chain CDR1 that is or includes a sequence of SEQ ID NO: 1, a light chain CDR2 that is or includes a sequence of SEQ ID NO: 2 and a light chain CDR3 that is or includes a sequence of SEQ ID NO: 3 or 4. In some examples, an anti-4-1BB antibody or antigen-binding antibody fragment includes each of: a light chain CDR1 that is or includes a sequence of SEQ ID NO: 1, a light chain CDR2 that is or includes a sequence of SEQ ID NO: 2 and a light chain CDR3 that is or includes a sequence of SEQ ID NO: 3 or 4.

[0006] In some examples, an anti-4-1BB antibody or antigen-binding antibody fragment includes a heavy chain variable domain that includes a heavy chain CDR1 that is or includes a sequence of SEQ ID NO: 5, a heavy chain CDR2 that is or includes a sequence of SEQ ID NO: 6 and a heavy chain CDR3 that is or includes a sequence of SEQ ID NO: 7 or 8, and/or a light chain variable domain that includes a light chain CDR1 that is or includes a sequence of SEQ ID NO: 1, a light chain CDR2 that is or includes a sequence of SEQ ID NO: 2 and a light chain CDR3 that is or includes a sequence of SEQ ID NO: 4.

[0006a] In one aspect, the present disclosure refers to an anti-4-1BB antibody or antigen-binding fragment thereof, comprising:

- (a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and
- (b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, wherein the antibody fragment is a Fv fragment, a disulfide-bonded Fv fragment, a single domain antibody, nanobody, or a diabody.

[0007] In some examples, an anti-4-1BB antibody or antigen-binding antibody fragment includes a heavy chain variable domain that includes a heavy chain framework 1 (FR1) region comprising a sequence of SEQ ID NO: 16 or 17. In some examples, an anti-4-1BB antibody or antigen-binding antibody fragment includes a heavy chain variable domain that

comprises or delivers an anti-4-1BB antibody or antigen-binding fragment. In some examples, the present disclosure provides methods of treating a subject in need thereof, the method comprising administering to the subject a composition that comprises or delivers a nucleic acid and/or vector encoding an anti-4-1BB antibody or antigen-binding fragment. In some examples, a subject has or is at risk for developing cancer. In one aspect, the present disclosure refers to use of a composition that comprises or delivers an anti-4-1BB antibody or antigen-binding fragment thereof comprising: (a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and (b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, any one of the nucleic acids disclosed herein or any one of the recombinant vectors disclosed herein, in the manufacture of a medicament for treating a subject in need thereof, wherein the subject has or is at risk for developing cancer.

[0021] In some examples, the present disclosure provides methods of inducing an immune response in a subject in need thereof, the method comprising administering to the subject a composition that comprises or delivers an anti-4-1BB antibody or antigen-binding fragment. In some examples, the present disclosure provides methods of inducing an immune response in a subject in need thereof, the method comprising administering to the subject a composition that comprises or delivers a nucleic acid and/or vector encoding an anti-4-1BB antibody or antigen-binding fragment. In some examples, a subject has or is at risk for developing cancer. In one aspect, the present disclosure refers to use of a composition that comprises or delivers an anti-4-1BB antibody or antigen-binding fragment thereof comprising: (a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and (b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, any one of the nucleic acids disclosed herein or any one of the recombinant vectors disclosed herein, in the manufacture of a medicament for inducing an immune response in a subject in need thereof.

[0022] In some examples, the present disclosure provides methods of enhancing an immune response in a subject in need thereof, the method comprising administering to the subject a composition that comprises or delivers an anti-4-1BB antibody or antigen-binding fragment. In some examples, the present disclosure provides methods of enhancing an immune response in a subject in need thereof, the method comprising administering to the subject a composition that comprises or delivers a nucleic acid and/or vector encoding an anti-4-1BB antibody or antigen-binding fragment. In some examples, a subject has or is at risk for developing cancer. In one aspect, the present disclosure refers to use of a composition that comprises or delivers an anti-4-1BB antibody or antigen-binding fragment thereof comprising: (a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and (b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, any one of the nucleic acids disclosed herein or any one of the recombinant vectors disclosed herein, in the manufacture of a medicament for enhancing an immune response or increasing the activity of an immune cell in a subject in need thereof, wherein the subject has or is at risk for developing cancer

[0023] In some examples, a cancer to be treated by a method of the present disclosure in a subject is selected from a bladder cancer, breast cancer, cervical cancer, colon cancer, endometrial cancer, esophageal cancer, fallopian tube cancer, gall bladder cancer, gastrointestinal cancer, head and neck cancer, hematological cancer, laryngeal cancer, liver cancer, lung cancer, lymphoma, melanoma, mesothelioma, ovarian cancer, primary peritoneal cancer, salivary gland cancer, sarcoma, stomach cancer, thyroid cancer, pancreatic cancer, and prostate cancer.

[0024] In some examples, a composition comprises or delivers an anti-human 4-1BB antibody of the present disclosure or an antigen-binding fragment thereof at a dose of 0.01 mg/kg to 100 mg/kg. In some examples, a composition comprises or delivers an anti-human 4-1BB

a subject in need thereof. In some examples, such a method includes (i) providing or obtaining a measurement of secreted IFN-gamma in a biological sample from the subject, wherein the subject has been administered a composition that comprises or delivers an amount of an anti-4-1BB antibody or antigen-binding fragment described herein; and (ii) comparing the measurement of secreted IFN-gamma to a reference value, where if the measurement of secreted IFN-gamma is higher or lower than the reference value, adjusting the amount of an anti-4-1BB antibody or antigen binding fragment thereof to be administered, thereby determining a dose for therapeutic treatment of a subject. In some examples, a reference value comprises an index value which includes a value derived from one or more healthy subjects, a value derived from one or more cancer diagnosed subject or a value derived from a cancer risk prediction algorithm. In some examples, a biological sample is a sample of whole blood, plasma, or serum. In some examples, a subject has or is at risk for developing cancer. In some examples, a cancer is selected from a bladder cancer, breast cancer, cervical cancer, colon cancer, endometrial cancer, esophageal cancer, fallopian tube cancer, gall bladder cancer, gastrointestinal cancer, head and neck cancer, hematological cancer, laryngeal cancer, liver cancer, lung cancer, lymphoma, melanoma, mesothelioma, ovarian cancer, primary peritoneal cancer, salivary gland cancer, sarcoma, stomach cancer, thyroid cancer, pancreatic cancer, and prostate cancer. In one aspect, the present disclosure refers to a method of determining a dose of an anti-4-1BB antibody or antigen binding fragment thereof for therapeutic treatment of a subject in need thereof, comprising: (a) providing or obtaining a measurement of secreted IFN-gamma in a biological sample from the subject, wherein the subject has been administered a composition that comprises or delivers an amount of an anti-4-1BB antibody or antigen-binding fragment thereof comprising: (A) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and (B) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4; and (b) comparing the measurement of secreted IFN-gamma to a reference value, wherein if the measurement of secreted IFN-gamma is higher or lower than the reference value, adjusting the amount of an anti-4-1BB antibody or antigen binding fragment thereof to be administered, thereby determining a dose for therapeutic treatment of a subject, wherein the reference value comprises an index value which includes a value derived from one or more healthy subjects, a value derived from one or more cancer diagnosed subject or a value derived from a cancer risk prediction algorithm.

[0030] In some examples, the present disclosure provides methods for increasing secretion of IFN- γ by a cell *in vivo* or *in vitro* that include: contacting the cell with an anti-4-1BB antibody or antigen-binding fragment described herein. In one aspect, the present disclosure refers to an *in vitro* method for increasing secretion of IFN- γ by a cell, the method comprising: (a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and (b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4. In one aspect, the present disclosure refers to use of an anti-4-1BB antibody or antigen-binding fragment thereof comprising: (a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and (b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, in the manufacture of an anti-cancer medicament for increasing secretion of IFN- γ by a cell.

[0031] In some examples, the present disclosure provides methods *ex vivo* proliferation or isolation of activated T cells that include: contacting a population of T cells with an anti-4-1BB antibody or antigen-binding fragment described herein, thereby increasing proliferation of activated T cells. In one aspect, the present disclosure refers to a method for *ex vivo* proliferation or isolation of activated T cells, the method comprising: contacting a population of T cells with an anti-4-1BB antibody or antigen-binding fragment thereof comprising: (a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and (b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, thereby increasing proliferation of activated T cells.

[0032] In some examples, the present disclosure provides methods for isolating antigen-specific activated T cells that include one or more steps of: (a) culturing peripheral blood mononuclear cells (PBMC) in a medium together with a peptide of an epitope of interest and IL-2; (b) inducing 4-1BB expression in the cultured cells by adding the peptide of the epitope of interest; (c) contacting the cultured cells with a surface coated with an anti-4-1BB antibody or antigen-binding fragment described herein, wherein cultured cells expressing 4-1BB adhere to

[0035] The present disclosure provides various technologies related to making or manufacturing anti-human 4-1BB antibodies and/or fragments thereof as described herein and/or compositions containing said antibodies or fragments thereof.

[0036] As used in this application, the terms “about” and “approximately” are used as equivalents. Any citations to publications, patents, or patent applications herein are incorporated by reference in their entirety. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

CLAIMS

What is claimed is:

1. A nucleic acid molecule encoding an anti-4-1BB antibody or antigen-binding fragment thereof, comprising:
 - (a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and
 - (b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4.
2. The nucleic acid molecule of claim 1, wherein the antibody or antigen-binding fragment comprises:
 - (a) a heavy chain framework 1 (FR1) region comprising a sequence of SEQ ID NO: 16 or 17; and/or
 - (b) a heavy chain framework 3 (FR3) region comprising a sequence of any one of SEQ ID NOs: 18-20.
3. The nucleic acid molecule of claim 1 or 2, wherein the antibody or antigen-binding fragment comprises any one of the following:
 - (a) a heavy chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 11-14;
 - (b) a light chain variable domain comprising a sequence at least 98% identical to a sequence of SEQ ID NO: 10; or
 - (c) a heavy chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 11-14 and a light chain variable domain comprising a sequence at least 98% identical to a sequence of SEQ ID NO: 10.
4. The nucleic acid molecule of any one of claims 1-3, wherein the antibody or antigen-binding fragment comprises any one of the following:
 - (a) a heavy chain variable domain comprising a sequence selected from SEQ ID NOs: 11-14;

- (b) a light chain variable domain comprising a sequence of SEQ ID NO: 10; or
- (c) a heavy chain variable domain comprising a sequence selected from SEQ ID NOs: 11-14 and a light chain variable domain comprising a sequence of SEQ ID NO: 10.
5. The nucleic acid molecule of any one of claims 1-4, wherein the antibody or antigen-binding fragment has a binding affinity (K_D) for a human 4-1BB molecule of 1×10^{-7} to 1×10^{-12} M.
6. The nucleic acid molecule of any one of claims 1-5, wherein the antibody or antigen-binding fragment binds to an epitope within the extracellular domain of human 4-1BB polypeptide.
7. The nucleic acid molecule of claim 6, wherein the binding to an epitope within the extracellular domain of human 4-1BB is abrogated by one or more mutations at positions N30, D38, N39, and R41 of SEQ ID NO: 44.
8. The nucleic acid molecule of any one of claims 1-7, wherein the antibody or antigen-binding fragment fails to bind or weakly binds a canine 4-1BB polypeptide.
9. The nucleic acid molecule of any one of claims 1-8, wherein the antibody or antigen-binding fragment is or comprises a humanized antibody.
10. The nucleic acid molecule of any one of claims 1-9, wherein the antibody includes an immunoglobulin constant domain, wherein the constant domain is selected from an IgG1, an IgG2, an IgG4, an IgA, an IgE, an IgM, and an IgD.
11. The nucleic acid molecule of any one of claims 1-10, wherein the antibody is or comprises a human IgG1.
12. The nucleic acid molecule of claim 11, wherein the IgG1 is or comprises a sequence that is at least 95% identical to SEQ ID NO: 22 or 23.

13. The nucleic acid molecule of any one of claims 1-12, wherein the antibody or antigen-binding fragment is a monoclonal antibody.

14. The nucleic acid molecule of any one of claims 1-10, wherein the antibody fragment is a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a Fv fragment, a disulfide-bonded Fv fragment, a scFv fragment, a single domain antibody, nanobody, or a diabody.

15. An anti-4-1BB antibody or antigen-binding fragment thereof, comprising:

(a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and

(b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, wherein the antibody fragment is a Fv fragment, a disulfide-bonded Fv fragment, a single domain antibody, nanobody, or a diabody.

16. A recombinant vector comprising the nucleic acid molecule of any one of claims 1-14.

17. A host cell comprising the recombinant vector of claim 16 and/or the nucleic acid molecule of any one of claims 1-14.

18. The host cell of claim 17, wherein the host cell is selected from a bacterial, yeast, insect or mammalian cell.

19. The host cell of claim 18, wherein the host cell is selected from the group consisting of *E.coli*, *P.pastoris*, Sf9, COS, HEK293, CHO and a mammalian lymphocyte.

20. A pharmaceutical composition comprising:

(a) the anti-4-1BB antibody or antigen-binding fragment of claim 15, the nucleic acid molecule of any one of claims 1-14, the recombinant vector of claim 16, or the host cell of claim 17; and

(b) a pharmaceutically acceptable carrier.

21. Use of a composition that comprises or delivers an anti-4-1BB antibody or antigen-binding fragment thereof comprising:

(a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and

(b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, a nucleic acid of any one of claims 1-14 or a recombinant vector of claim 16, in the manufacture of a medicament for treating a subject in need thereof, wherein the subject has or is at risk for developing cancer.

22. Use of a composition that comprises or delivers an anti-4-1BB antibody or antigen-binding fragment thereof comprising:

(a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and

(b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, a nucleic acid of any one of claims 1-14 or a recombinant vector of claim 16, in the manufacture of a medicament for inducing an immune response in a subject in need thereof.

23. Use of a composition that comprises or delivers an anti-4-1BB antibody or antigen-binding fragment thereof comprising:

(a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and

(b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, a nucleic acid of any one of claims 1-14 or a recombinant vector of claim 16, in the manufacture of

a medicament for enhancing an immune response or increasing the activity of an immune cell in a subject in need thereof, wherein the subject has or is at risk for developing cancer.

24. The use of claim 22, wherein the subject has or is at risk for developing cancer.

25. The use of any one of claims 21, 23, and 24, where in the cancer is selected from a bladder cancer, breast cancer, cervical cancer, colon cancer, endometrial cancer, esophageal cancer, fallopian tube cancer, gall bladder cancer, gastrointestinal cancer, head and neck cancer, hematological cancer, laryngeal cancer, liver cancer, lung cancer, lymphoma, melanoma, mesothelioma, ovarian cancer, primary peritoneal cancer, salivary gland cancer, sarcoma, stomach cancer, thyroid cancer, pancreatic cancer, and prostate cancer.

26. The use of any one of claims 21-25, wherein the subject is one that has been administered or will be administered one or more additional anticancer therapies selected from ionizing radiation, a chemotherapeutic agent, an antibody agent, and a cell-based therapy, such that the subject receives treatment with both.

27. The use of claim 26, wherein the one or more additional anticancer therapies comprise an immune checkpoint inhibitor, IL-12, GM-CSF, an anti-CD4 agent, cisplatin, fluorouracil, doxorubicin, irinotecan, paclitaxel, or cyclophosphamide.

28. The use of any one of claims 21-25, wherein the subject is one that has been administered or will be administered a composition comprising an immune checkpoint inhibitor, such that the subject receives treatment with both.

29. The use of claim 27 or 28, wherein the immune checkpoint inhibitor is an agent that inhibits PD-1 signaling.

30. The use of claim 29, wherein the agent that inhibits PD-1 signaling is a PD-1 antibody.

31. A method of determining a dose of an anti-4-1BB antibody or antigen binding fragment thereof for therapeutic treatment of a subject in need thereof, comprising;

(a) providing or obtaining a measurement of secreted IFN-gamma in a biological sample from the subject, wherein the subject has been administered a composition that comprises or delivers an amount of an anti-4-1BB antibody or antigen-binding fragment thereof comprising:

(A) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and

(B) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4; and

(b) comparing the measurement of secreted IFN-gamma to a reference value, wherein if the measurement of secreted IFN-gamma is higher or lower than the reference value, adjusting the amount of an anti-4-1BB antibody or antigen binding fragment thereof to be administered, thereby determining a dose for therapeutic treatment of a subject,

wherein the reference value comprises an index value which includes a value derived from one or more healthy subjects, a value derived from one or more cancer diagnosed subject or a value derived from a cancer risk prediction algorithm.

32. The method of claim 31, wherein the biological sample is a sample of whole blood, plasma, or serum.

33. The method of claim 31 or 32, wherein the subject has or is at risk for developing cancer.

34. The method of claim 33, where in the cancer is selected from a bladder cancer, breast cancer, cervical cancer, colon cancer, endometrial cancer, esophageal cancer, fallopian tube cancer, gall bladder cancer, gastrointestinal cancer, head and neck cancer, hematological cancer, laryngeal cancer, liver cancer, lung cancer, lymphoma, melanoma, mesothelioma, ovarian cancer, primary peritoneal cancer, salivary gland cancer, sarcoma, stomach cancer, thyroid cancer, pancreatic cancer, and prostate cancer.

35. An *in vitro* method for increasing secretion of IFN- γ by a cell, the method comprising:
contacting the cell with an anti-4-1BB antibody or antigen-binding fragment thereof comprising:
(a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and
(b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4.
36. Use of an anti-4-1BB antibody or antigen-binding fragment thereof comprising:
(a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and
(b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, in the manufacture of an anti-cancer medicament for increasing secretion of IFN- γ by a cell.
37. A method for *ex vivo* proliferation or isolation of activated T cells, the method comprising: contacting a population of T cells with an anti-4-1BB antibody or antigen-binding fragment thereof comprising:
(a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and
(b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, thereby increasing proliferation of activated T cells.
38. A method for isolating antigen-specific activated T cells, the method comprising:
(a) culturing peripheral blood mononuclear cells (PBMC) in a medium together with a peptide of an epitope of interest and IL-2;
(b) inducing 4-1BB expression in the cultured cells by adding the peptide of the epitope of interest;

(c) contacting the cultured cells with a surface coated with an anti-4-1BB antibody or antigen-binding fragment thereof comprising:

(a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 5, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 6 and a heavy chain CDR3 comprising a sequence of SEQ ID NO: 7 or 8; and

(b) a light chain CDR1 comprising a sequence of SEQ ID NO: 1, a light chain CDR2 comprising a sequence of SEQ ID NO: 2 and a light chain CDR3 comprising a sequence of SEQ ID NO: 4, wherein cultured cells expressing 4-1BB adhere to the coated surface; and

(d) removing unattached cells, thereby isolating antigen-specific activated T cells.

39. The method of claim 38, wherein the activated T cells are CD8⁺ T cells.

40. Use a therapeutically effective amount of activated T cells produced by the method of any one of claims 37-39 in the manufacture of a medicament for treating or preventing cancer in a subject in need thereof.

41. The use of any one of claims 21-23 and 36, or the method of any one of claims 31, 35, 37 and 38, wherein the antibody or antigen-binding fragment comprises:

(a) a heavy chain framework 1 (FR1) region comprising a sequence of SEQ ID NO: 16 or 17; and/or

(b) a heavy chain framework 3 (FR3) region comprising a sequence of any one of SEQ ID NOs: 18-20.

42. The use of any one of claims 21-23, 36 and 41, or the method of any one of claims 31, 35, 37, 38 and 41, wherein the antibody or antigen-binding fragment comprises any one of the following:

(a) a heavy chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 11-14;

(b) a light chain variable domain comprising a sequence at least 98% identical to a sequence of SEQ ID NO: 10; or

(c) a heavy chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 11-14 and a light chain variable domain comprising a sequence at least 98% identical to a sequence of SEQ ID NO: 10.

43. The use of any one of claims 21-23, 36, 41 and 42, or the method of any one of claims 31, 35, 37, 38, 41 and 42, wherein the antibody or antigen-binding fragment comprises any one of the following:

- (a) a heavy chain variable domain comprising a sequence selected from SEQ ID NOs: 11-14;
- (b) a light chain variable domain comprising a sequence of SEQ ID NO: 10; or
- (c) a heavy chain variable domain comprising a sequence selected from SEQ ID NOs: 11-14 and a light chain variable domain comprising a sequence of SEQ ID NO: 10.

44. The use of any one of claims 21-23, 36 and 41-43, or the method of any one of claims 31, 35, 37, 38 and 41-43, wherein the antibody or antigen-binding fragment has a binding affinity (K_D) for a human 4-1BB molecule of 1×10^{-7} to 1×10^{-12} M.

45. The use of any one of claims 21-23, 36 and 41-44, or the method of any one of claims 31, 35, 37, 38 and 41-44, wherein the antibody or antigen-binding fragment binds to an epitope within the extracellular domain of human 4-1BB polypeptide.

46. The use or method of claim 45, wherein the binding to an epitope within the extracellular domain of human 4-1BB is abrogated by one or more mutations at positions N30, D38, N39, and R41 of SEQ ID NO: 44.

47. The use of any one of claims 21-23, 36, and 41-46, or the method of any one of claims 31, 35, 37, 38 and 41-46, wherein the antibody or antigen-binding fragment fails to bind or weakly binds a canine 4-1BB polypeptide.

48. The use of any one of claims 21-23, 36, and 41-47, or the method of any one of claims 31, 35, 37, 38 and 41-47, wherein the antibody or antigen-binding fragment is or comprises a humanized antibody.

49. The use of any one of claims 21-23, 36, and 41-48, or the method of any one of claims 31, 35, 37, 38 and 41-48, wherein the antibody includes an immunoglobulin constant domain, wherein the constant domain is selected from an IgG1, an IgG2, an IgG4, an IgA, an IgE, an IgM, and an IgD.

50. The use of any one of claims 21-23, 36, and 41-49, or the method of any one of claims 31, 35, 37, 38 and 41-49, wherein the antibody is or comprises a human IgG1.

51. The use or method of claim 50, wherein the IgG1 is or comprises a sequence that is at least 95% identical to SEQ ID NO: 22 or 23.

52. The use of any one of claims 21-23, 36, and 41-51, or the method of any one of claims 31, 35, 37, 38 and 41-51, wherein the antibody or antigen-binding fragment is a monoclonal antibody.

53. The use of any one of claims 21-23, 36, and 41-52, or the method of any one of claims 31, 35, 37, 38 and 41-52, wherein the antibody fragment is a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a Fv fragment, a disulfide-bonded Fv fragment, a scFv fragment, a single domain antibody, humabody, nanobody, or a diabody.