

ANTI-HUMAN 4-1BB ANTIBODIES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of US Patent Application No. 62/443,281, filed on January 06, 2017, the disclosure of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Cancer remains one of the leading causes of death in the world. Recent statistics report that 13% of the world population dies from cancer. According to estimates from the International Agency for Research on Cancer (IARC), in 2012 there were 14.1 million new cancer cases and 8.2 million cancer deaths worldwide. By 2030, the global burden is expected to grow to 21.7 million new cancer cases and 13 million cancer deaths due to population growth and aging and exposure to risk factors such as smoking, unhealthy diet and physical inactivity. Further, pain and medical expenses for cancer treatment cause reduced quality of life for both cancer patients and their families. It is apparent that, above all, cancer is a disease for which it is necessary to urgently find improved treatment methods.

SUMMARY

[0003] The present disclosure provides, among other things, antibodies and fragments thereof that bind to a human 4-1BB polypeptide. In some [aspectexamples](#), provided anti-human 4-1BB antibodies and fragments thereof are variants of a reference anti-human 4-1BB antibody in that they contain one or more particular structural features that are not found in the reference anti-human 4-1BB antibody. The present disclosure encompasses a recognition that provided variant anti-human 4-1BB antibodies have improved properties relative to a reference anti-human 4-1BB antibody lacking one or more structural features described herein. In some [embodimentexamples](#), provided anti-human 4-1BB antibodies and fragments thereof have one or more improved properties, such as, for example, improved binding affinity, improved induction of T cell proliferation (e.g., proliferation of CD8⁺ T cells), increased ability to induce IFN γ production by

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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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, humabody, nanobody, or a diabody.

[0007] In some [embodimentexamples](#), 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 [embodimentexamples](#), an anti-4-1BB antibody or antigen-binding antibody fragment includes a heavy chain variable domain that

includes a heavy chain framework 3 (FR3) region comprising a sequence of any one of SEQ ID NOs: 18-20. In some [embodimentexamples](#), 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 and a heavy chain framework 3 (FR3) region comprising a sequence of any one of SEQ ID NOs: 18-20.

[0008] In some [embodimentexamples](#), an anti-4-1BB antibody or antigen-binding antibody fragment includes substantial homology to an antibody or antibody fragment that includes a heavy chain variable domain that is or includes a sequence selected from SEQ ID NOs: 11-14. In some [embodimentexamples](#), an anti-4-1BB antibody or antigen-binding antibody fragment includes a heavy chain variable domain that is or includes a sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4% or 99.5% identical to a sequence selected from SEQ ID NOs: 11-14. In some [embodimentexamples](#), an anti-4-1BB antibody or antigen-binding antibody fragment includes a heavy chain variable domain that is or includes a sequence selected from SEQ ID NOs: 11-14.

[0009] In some [embodimentexamples](#), an anti-4-1BB antibody or antigen-binding antibody fragment includes substantial homology to an antibody or antibody fragment that includes a light chain variable domain that is or includes a sequence of SEQ ID NO: 9 or 10. In some [embodimentexamples](#), an anti-4-1BB antibody or antigen-binding antibody fragment includes a light chain variable domain that is or includes a sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4% or 99.5% identical to a sequence of SEQ ID NO: 9 or 10. In some [embodimentexamples](#), an anti-4-1BB antibody or antigen-binding antibody fragment includes a light chain variable domain that is or includes a sequence of SEQ ID NO: 9 or 10.

[0010] In some [embodimentexamples](#), an anti-4-1BB antibody or antigen-binding antibody fragment includes substantial homology to an antibody or antibody fragment that includes a heavy chain variable domain that is or includes a sequence selected from SEQ ID NOs: 11-14 and a light chain variable domain that is or includes a sequence of SEQ ID NO: 10. In some [embodimentexamples](#), an anti-4-1BB antibody or antigen-binding antibody fragment includes a heavy chain variable domain that is or includes a sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4% or 99.5% identical to a sequence selected from SEQ ID NOs: 11-14 and a light chain variable domain that is or includes a sequence at least

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4% or 99.5% identical to a sequence of SEQ ID NO: 10. In some [embodimentexamples](#), an anti-4-1BB antibody or antigen-binding antibody fragment includes a heavy chain variable domain that is or includes a sequence selected from SEQ ID NOs: 11-14 and a light chain variable domain that is or includes a sequence of SEQ ID NO: 10.

[0011] In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof is an agonistic antibody. In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof is characterized as having superior agonistic activity than a humanized anti-human 4-1BB antibody 94G1 (i.e., an antibody including light chain and heavy chain variable domains of SEQ ID NOs: 9 and 11, respectively). In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof is characterized as having improved binding affinity than a humanized anti-human 4-1BB antibody 94G1 (i.e., an antibody including light chain and heavy chain variable domains of SEQ ID NOs: 9 and 11, respectively).

[0012] In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof is or comprises a humanized antibody. In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof includes a human immunoglobulin constant domain, wherein the constant domain is selected from an IgG1 or a variant thereof, an IgG2 or a variant thereof, an IgG4 or a variant thereof, an IgA or a variant thereof, an IgE or a variant thereof, an IgM or a variant thereof, and an IgD or a variant thereof. In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof is or comprises a human IgG1. In some [embodimentexamples](#), an IgG1 is or comprises a sequence that is at least 95% identical to SEQ ID NO: 22 or 23.

[0013] In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof is a monoclonal antibody.

[0014] In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof is a full length antibody. In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof 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.

[0015] In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof has a binding affinity (K_D) for a human 4-1BB molecule of 1×10^{-7} to 1×10^{-12} M. In some

[embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof has a binding affinity (K_D) for a human 4-1BB molecule of 1×10^{-8} to 1×10^{-12} M. In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof has a binding affinity (K_D) for a human 4-1BB molecule of 1×10^{-9} to 1×10^{-12} M. In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof has a binding affinity (K_D) for a human 4-1BB molecule of 1×10^{-10} to 1×10^{-12} M.

[0016] In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof binds to an epitope within the extracellular domain of a human 4-1BB polypeptide. In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof binds to an epitope within the extracellular domain of human 4-1BB. In some [embodimentexamples](#), binding of a provided anti-human 4-1BB antibody or fragment thereof is abrogated by one or more mutations at positions N30, D38, N39, and R41 of SEQ ID NO: 44.

[0017] In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof fails to bind or weakly binds a non-primate 4-1BB polypeptide. In some [embodimentexamples](#), a provided anti-human 4-1BB antibody or fragment thereof fails to bind or weakly binds a canine 4-1BB polypeptide.

[0018] In some [embodimentexamples](#), the present disclosure provides nucleic acid molecules encoding an anti-4-1BB antibody or antigen-binding fragment. In some [embodimentexamples](#), the present disclosure provides vectors that include a nucleic acid molecule encoding an anti-4-1BB antibody or antigen-binding fragment. In some [embodimentexamples](#), the present disclosure provides host cells that include a vector and/or nucleic acid molecule encoding an anti-4-1BB antibody or antigen-binding fragment. In some [embodimentexamples](#), a host cell is selected from a bacterial, yeast, insect or mammalian cell. In some [embodimentexamples](#), a is selected from the group consisting of *E.coli*, *P.pastoris*, Sf9, COS, HEK293, CHO and a mammalian lymphocyte.

[0018a] In one aspect, the present disclosure refers to 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.

[0018b] In one aspect, the present disclosure refers to a recombinant vector comprising any one of the nucleic acid molecules disclosed herein.

[0018c] In one aspect, the present disclosure refers to a host cell comprising any one of the recombinant vectors disclosed herein and/or any one of the nucleic acid molecules disclosed herein.

[0019] In some [embodimentexamples](#), the present disclosure provides pharmaceutical compositions that include an anti-4-1BB antibody or antigen-binding fragment and a pharmaceutically acceptable carrier. In some [embodimentexamples](#), the present disclosure provides pharmaceutical compositions that include a nucleic acid and/or vector encoding an anti-4-1BB antibody or antigen-binding fragment and a pharmaceutically acceptable carrier. In one aspect, the present disclosure refers to a pharmaceutical composition comprising: (a) the anti-4-1BB antibody or antigen-binding fragment disclosed herein, any one of the nucleic acid molecules disclosed herein, any one of the recombinant vector disclosed herein, or any one of the host cells disclosed herein; and (b) a pharmaceutically acceptable carrier.

[0020] In some [embodimentexamples](#), 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 an anti-4-1BB antibody or antigen-binding fragment. In some [embodimentexamples](#), 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 [embodimentexamples](#), 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.

[0021] In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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.

[0023] In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), a composition comprises or delivers an anti-human 4-1BB

antibody or an antigen-binding fragment thereof at a dose of about 0.01 mg/kg, 0.025 mg/kg, 0.05 mg/kg, 0.075 mg/kg, 0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 0.75 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 8 mg/kg, 10 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 40 mg/kg, 50 mg/kg, 50 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, or 100 mg/kg.

[0025] In some [embodimentexamples](#), anti-human 4-1BB antibodies and/or fragments thereof and/or compositions comprising the same are characterized by inducing increased T cell proliferation (e.g., CD8⁺ T cell proliferation) and/or increased IFN γ secretion by T cells (e.g., CD8⁺ T cells) in a subject.

[0026] In some [embodimentexamples](#), the present disclosure provides methods that include administering to the subject a composition that comprises or delivers an anti-4-1BB antibody or antigen-binding fragment to a subject that has been administered or will be administered one or more additional anticancer therapies. In some [embodimentexamples](#), the present disclosure provides methods that include administering to the subject a composition that comprises or delivers an anti-4-1BB antibody or antigen-binding fragment to a subject that has been administered or will be administered one or more of ionizing radiation, a chemotherapeutic agent, an antibody agent, and a cell-based therapy, such that the subject receives treatment with both.

[0027] In some [embodimentexamples](#), the present disclosure provides methods that include administering to the subject a composition that comprises or delivers an anti-4-1BB antibody or antigen-binding fragment to a subject that has been administered or will be administered one or more of an immune checkpoint inhibitor, IL-12, GM-CSF, an anti-CD4 agent, fluorouracil, doxorubicin, irinotecan, paclitaxel, cisplatin, or cyclophosphamide.

[0028] In some [embodimentexamples](#), the present disclosure provides methods that include administering to the subject a composition that comprises or delivers an anti-4-1BB antibody or antigen-binding fragment to a subject that has been administered or will be administered a composition comprising an immune checkpoint inhibitor, such that the subject receives treatment with both. In some [embodimentexamples](#), an immune checkpoint inhibitor is an agent that inhibits PD-1 signaling. In some [embodimentexamples](#), an agent that inhibits PD-1 signaling is an anti-PD-1 antibody. In some [embodimentexamples](#), an anti-PD-1 antibody is nivolumab, pembrolizumab, atezolizumab, durvalumab, or avelumab.

[0029] In some [embodimentexamples](#), the present disclosure provides methods of determining a dose of an anti-4-1BB antibody or antigen binding fragment thereof for therapeutic treatment of

a subject in need thereof. In some [embodimentexamples](#), 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 [embodimentexample](#), 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 [embodimentexamples](#), a biological sample is a sample of whole blood, plasma, or serum. In some [embodimentexamples](#), a subject has or is at risk for developing cancer. In some [embodimentexamples](#), 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.

[0030] In some [embodimentexamples](#), 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- \$\gamma\$ 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 a medicament for increasing secretion of IFN- \$\gamma\$ by a cell.](#)

[0031] In some [embodimentexamples](#), 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 [embodimentexamples](#), 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

the coated surface; and (d) removing unattached cells, thereby isolating antigen-specific activated T cells. In some [embodimentexamples](#), activated T cells are CD8⁺ T cells. [In one aspect, the present disclosure refers to 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.](#)

[0033] In some [embodimentexamples](#), the present disclosure provides methods for treating or preventing cancer in a subject in need thereof that includes administering to the subject a composition that includes a therapeutically effective amount of activated T cells produced by any of the method described herein. In some [embodimentexamples](#), 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 some [embodimentexamples](#), a composition includes least 10⁹, at least 10¹⁰ cells, or more than 10¹⁰ activated T cells. In some [embodimentexamples](#), activated T cells are CD8⁺ T cells. [In one aspect, the present disclosure refers to use of a therapeutically effective amount of activated T cells produced by any one of the methods disclosed herein in the manufacture of a medicament for treating or preventing cancer in a subject in need thereof.](#)

[0034] Also provided, among other things, are technologies for characterizing anti-human 4-1BB antibodies and/or fragments thereof as described herein and/or compositions comprising the same. In some [embodimentexamples](#), provided are methods for characterizing anti-human 4-1BB antibodies and/or fragments thereof and/or compositions comprising the same binding to AML cells (e.g., HL60). In some [embodimentexamples](#), provided are methods for characterizing anti-human 4-1BB antibodies and/or fragments thereof and/or compositions comprising the same are by ELISA, immunohistochemistry, Biacore binding assays, mass spectrometry, isoelectric focusing (IEF) chromatography, and/or western blot.

[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.

[0035a] In one aspect, the present disclosure refers to a polypeptide 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 a nucleic acid molecule encoding a polypeptide disclosed in this aspect. In one aspect, the present disclosure refers to a recombinant vector comprising the nucleic acid molecule disclosed in this aspect. In one aspect, the present disclosure refers to a host cell comprising the nucleic acid molecule disclosed in this aspect. In one aspect, the present disclosure refers to a pharmaceutical composition comprising: (a) a polypeptide disclosed in this aspect; and (b) a pharmaceutically acceptable carrier. In one aspect, the present disclosure refers to use of a composition that comprises or delivers a polypeptide disclosed in this aspect in the manufacture of a medicament for treating a subject in need thereof. In one aspect, the present disclosure refers to use of a composition that comprises or delivers a polypeptide disclosed in this aspect in the manufacture of a medicament for inducing an immune response in a subject in need thereof. In one aspect, the present disclosure refers to use of a composition that comprises or delivers a polypeptide disclosed in this aspect 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. In one aspect, the present disclosure refers to an *in vitro* method for increasing secretion of IFN- γ by a cell, the method comprising: contacting the cell with a polypeptide disclosed in this aspect. In one aspect, the present disclosure refers to use of the cell with a polypeptide disclosed in this aspect in the manufacture of a medicament for increasing secretion of IFN- γ by a cell. 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 a polypeptide disclosed in this aspect, thereby increasing proliferation of activated T cells. In one aspect, the present disclosure refers to 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 a polypeptide disclosed in this aspect, wherein cultured cells expressing 4-1BB adhere to the coated surface; and (d) removing unattached cells, thereby isolating antigen-specific activated T cells. In one aspect, the present disclosure refers to use of the activated T cells produced by the method disclosed in this aspect in the manufacture of a medicament for treating or preventing cancer in a subject in need 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.

[0037] Other features, objects, and advantages of the present [inventiondisclosure](#) are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating [embodimentexamples](#) of the present [inventiondisclosure](#), is given by way of illustration only, not limitation. Various changes and modifications within the scope of the [inventiondisclosure](#) will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWING

[0038] The Drawing included herein, which is comprised of the following Figures, is for illustration purposes only and not for limitation. The foregoing and other objects, aspects, features, and advantages of the present disclosure will become more apparent and better understood by referring to the following description taken in conjunction with the accompanying figures in which:

[0039] **FIG. 1A** depicts human 4-1BB extracellular domain (ECD) constructs. At the top is a schematic of a full length 4-1BB ECD (167 amino acids), and below is shown various fragments of a 4-1BB ECD: R1 (1-55 aa), R2 (56-110 aa), R3 (110-167 aa), R1.1 (1-45 aa), R1.2 (1-35 aa), R1.3 (11-55 aa), R1.4 (21-55 aa) R1.5 (1-25 aa) and R1.6 (1-30 aa). Each of these 4-1BB ECD constructs were fused with GST. **FIG. 1B** depicts a western blot showing binding of an exemplary humanized anti-4-1BB antibody to 4-1BB ECD fusion constructs as described in FIG. 1A. As shown, an exemplary humanized anti-4-1BB antibody binds to a full length 4-1BB ECD fusion polypeptide and to the R1 fusion polypeptide, but not to the R2 or R3 fusion polypeptides. Molecular size markers are indicated in kDa on the left.

[0040] **FIG. 2A** depicts an SDS-PAGE gel of whole cell extracts from cells expressing 4-1BB ECD fusion constructs. Fusion constructs as described above in FIG. 2A were expressed in *E. coli* BL21 cells induced with 1 mM IPTG, and whole cell extracts resolved by 12% SDS-PAGE. As shown, all fusion constructs (ECD, R1, R1.1, R1.2, R1.3, R1.4, R1.5, and R1.6) have robust protein expression. **FIG. 2B** depicts a western blot showing binding of an exemplary humanized anti-4-1BB antibody to full length 4-1BB ECD fusion polypeptide, and R1.1, R1.2, R1.3, and R1.6 fusion polypeptides, but not to the R1.4 or R1.5 fusion polypeptides. Immunoblots were performed with an exemplary anti-human 4-1BB antibody. Molecular size markers are indicated in kDa on the left.

CLAIMS

What is claimed is:

1. ~~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 anti-4-1BB antibody or antigen-binding fragment 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 anti-4-1BB antibody or antigen-binding fragment 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 anti-4-1BB antibody or antigen-binding fragment 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 anti-4-1BB antibody or antigen-binding fragment 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 anti-4-1BB antibody or antigen-binding fragment 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 anti-4-1BB antibody or antigen-binding fragment 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 anti-4-1BB antibody or antigen-binding fragment 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 anti-4-1BB antibody or antigen-binding fragment of any one of claims 1-8, wherein the antibody or antigen-binding fragment is or comprises a humanized antibody.~~

~~10. The anti-4-1BB antibody or antigen-binding fragment of any one of claims 1-9, wherein the antibody includes an immunoglobulin constant domain, wherein the constant domain is selected from an IgG1 or a variant thereof, an IgG2 or a variant thereof, an IgG4 or a variant thereof, an IgA or a variant thereof, an IgE or a variant thereof, an IgM or a variant thereof, and an IgD or a variant thereof.~~

~~11. The anti 4-1BB antibody or antigen-binding fragment of any one of claims 1-10, wherein the antibody is or comprises a human IgG1.~~

~~12. The anti 4-1BB antibody or antigen-binding fragment 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 anti 4-1BB antibody or antigen-binding fragment of any one of claims 1-12, wherein the antibody or antigen-binding fragment is a monoclonal antibody.~~

~~14. The anti 4-1BB antibody or antigen-binding fragment 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, humabody, nanobody, or a diabody.~~

15. A nucleic acid molecule encoding an anti-4-1BB antibody or antigen-binding fragment of any one of claims 1-14 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 or a variant thereof, an IgG2 or a variant thereof, an IgG4 or a variant thereof, an IgA or a variant thereof, an IgE or a variant thereof, an IgM or a variant thereof, and an IgD or a variant thereof.

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, humabody, 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, humabody, nanobody, or a diabody.

16. A recombinant vector comprising the nucleic acid molecule of ~~claim 15~~ 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~~claim 15~~.

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 ~~any one of claims 1-14~~claim 15, the nucleic acid molecule of any one of claims 1-14~~claim 16~~, the recombinant vector of claim ~~17~~16, or the host cell of claim 17; and

(b) a pharmaceutically acceptable carrier.

21. ~~A method of treating a subject in need thereof, the method comprising the steps of:~~
~~—administering to the subject—~~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~~of any one of claims 1-14~~, a nucleic acid of any one of claims 1-14~~claim 15~~ or a recombinant vector of claim 16, in the manufacture of a medicament for treating a subject in need thereof.

22. ~~A method of inducing an immune response in a subject in need thereof, the method comprising the steps of:~~

~~—administering to the subject—~~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 of any one of claims 1-14, a nucleic acid of any one of claims 1-14 claim 15 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. ~~A method of enhancing an immune response or increasing the activity of an immune cell in a subject in need thereof, the method comprising the steps of:~~

~~—administering to the subject~~ 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 of any one of claims 1-14, a nucleic acid of any one of claims 1-14 claim 15 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.

24. The ~~method-use~~ of any one of claims 21-23, wherein the subject has or is at risk for developing cancer.

25. The ~~method-use~~ of claim 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 method-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 method-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 method-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 method-use of claim 27 or 28, wherein the immune checkpoint inhibitor is an agent that inhibits PD-1 signaling.
30. The method-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~~of any one of claims 1-14~~; 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.

32. The method of claim 31, 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.

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

34. The method of any one of claims 31-33, wherein the subject has or is at risk for developing cancer.

35. The method of claim 34, 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.

36. An *in vitro* method for increasing secretion of IFN- γ by a cell ~~*in vivo* or *in vitro*~~, the method comprising: contacting the cell with an anti-4-1BB antibody or antigen-binding fragment ~~of 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: 3
~~any one of claims 1-14.~~

37. 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 a medicament for increasing secretion of IFN- γ by a cell.

3738. 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 of any one of claims 1-14,

thereby increasing proliferation of activated T cells.

3839. 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 of any one of claims 1-14, wherein cultured cells expressing 4-1BB adhere to the coated surface; and
(d) removing unattached cells, thereby isolating antigen-specific activated T cells.

3940. The method of claim 37-38 or 3839, wherein the activated T cells are CD8⁺ T cells.

4041. A method for treating or preventing cancer in a subject in need thereof, comprising: administering to the subject Use a therapeutically effective amount of activated T cells produced by the method of any one of claims 37-3938-40 in the manufacture of a medicament for treating or preventing cancer in a subject in need thereof.

42. The use of any one of claims 21-23 and 37, or the method of any one of claims 31, 36, 38 and 39, 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.

43. The use of any one of claims 21-23, 37 and 42, or the method of any one of claims 31, 36, 38, 39 and 42, 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.

44. The use of any one of claims 21-23, 37, 42 and 43, or the method of any one of claims 31, 36, 38, 39, 42 and 43, 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.

45. The use of any one of claims 21-23, 37 and 42-44, or the method of any one of claims 31, 36, 38, 39 and 42-44, 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.

46. The use of any one of claims 21-23, 37 and 42-45, or the method of any one of claims 31, 36, 38, 39 and 42-45, wherein the antibody or antigen-binding fragment binds to an epitope within the extracellular domain of human 4-1BB polypeptide.

47. The use or method of claim 46, 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.

48. The use of any one of claims 21-23, 37, and 42-47, or the method of any one of claims 31, 36, 38, 39 and 42-47, wherein the antibody or antigen-binding fragment fails to bind or weakly binds a canine 4-1BB polypeptide.

49. The use of any one of claims 21-23, 37, and 42-48, or the method of any one of claims 31, 36, 38, 39 and 42-48, wherein the antibody or antigen-binding fragment is or comprises a humanized antibody.

50. The use of any one of claims 21-23, 37, and 42-49, or the method of any one of claims 31, 36, 38, 39 and 42-49, wherein the antibody includes an immunoglobulin constant domain, wherein the constant domain is selected from an IgG1 or a variant thereof, an IgG2 or a variant

thereof, an IgG4 or a variant thereof, an IgA or a variant thereof, an IgE or a variant thereof, an IgM or a variant thereof, and an IgD or a variant thereof.

51. The use of any one of claims 21-23, 37, and 42-50, or the method of any one of claims 31, 36, 38, 39 and 42-50, wherein the antibody is or comprises a human IgG1.

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

53. The use of any one of claims 21-23, 37, and 42-52, or the method of any one of claims 31, 36, 38, 39 and 42-52, wherein the antibody or antigen-binding fragment is a monoclonal antibody.

54. The use of any one of claims 21-23, 37, and 42-53, or the method of any one of claims 31, 36, 38, 39 and 42-53, 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.

55. A polypeptide 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.

56. The polypeptide of claim 55, wherein the polypeptide further 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.

57. The polypeptide of claim 55, wherein the polypeptide 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.

58. The polypeptide of claim 55, wherein the polypeptide comprises:

(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.

59. The polypeptide of claim 55, wherein the polypeptide has a binding affinity (K_D) for a human 4-1BB molecule of 1×10^{-7} to 1×10^{-12} M.

60. The polypeptide of claim 55, wherein the polypeptide fails to bind or weakly binds a canine 4-1BB polypeptide.

61. The polypeptide of claim 55, wherein the polypeptide includes an immunoglobulin constant domain, wherein the constant domain is selected from an IgG1 or a variant thereof, an IgG2 or a variant thereof, an IgG4 or a variant thereof, an IgA or a variant thereof, an IgE or a variant thereof, an IgM or a variant thereof, and an IgD or a variant thereof.

62. A nucleic acid molecule encoding a polypeptide of claim 55.

63. A recombinant vector comprising the nucleic acid molecule of claim 62.

64. A host cell comprising the nucleic acid molecule of claim 62.

65. A pharmaceutical composition comprising:

(a) a polypeptide of claim 55; and

(b) a pharmaceutically acceptable carrier.

66. Use of a composition that comprises or delivers a polypeptide of claim 55 in the manufacture of a medicament for treating a subject in need thereof.

67. Use of a composition that comprises or delivers a polypeptide of claim 55 in the manufacture of a medicament for inducing an immune response in a subject in need thereof.

68. Use of a composition that comprises or delivers a polypeptide of claim 55 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.

69. The use of claim 68, wherein the subject has or is at risk for developing cancer.

70. An *in vitro* method for increasing secretion of IFN- γ by a cell, the method comprising: contacting the cell with a polypeptide of claim 55.

71. Use of the cell with a polypeptide of claim 55 in the manufacture of a medicament for increasing secretion of IFN- γ by a cell.

72. A method for *ex vivo* proliferation or isolation of activated T cells, the method comprising: contacting a population of T cells with a polypeptide of claim 55, thereby increasing proliferation of activated T cells.

73. 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 a polypeptide of claim 55, wherein cultured cells expressing 4-1BB adhere to the coated surface; and

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

74. Use of the activated T cells produced by the method of claim 73 in the manufacture of a medicament for treating or preventing cancer in a subject in need thereof.

75. The polypeptide of claim 55, wherein the polypeptide is a single-chain polypeptide.

76. The polypeptide of claim 55, wherein the polypeptide comprises two single-chain polypeptides.