

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 examples, 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 examples, 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 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 a first aspect, there is provided 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.

[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

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

[0008] In some examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, a provided anti-human 4-1BB antibody or fragment thereof is an agonistic antibody. In some examples, 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 examples, 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 examples, a provided anti-human 4-1BB antibody or fragment thereof is or comprises a humanized antibody. In some examples, 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 examples, a provided anti-human 4-1BB antibody or fragment thereof is or comprises a human IgG1. In some examples, an IgG1 is or comprises a sequence that is at least 95% identical to SEQ ID NO: 22 or 23.

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

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

examples 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 examples, 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 examples, 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 examples, 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 examples, a provided anti-human 4-1BB antibody or fragment thereof binds to an epitope within the extracellular domain of human 4-1BB. In some examples, 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 examples, a provided anti-human 4-1BB antibody or fragment thereof fails to bind or weakly binds a non-primate 4-1BB polypeptide. In some examples, a provided anti-human 4-1BB antibody or fragment thereof fails to bind or weakly binds a canine 4-1BB polypeptide.

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

[0019] In some examples, the present disclosure provides pharmaceutical compositions that include an anti-4-1BB antibody or antigen-binding fragment and a pharmaceutically acceptable carrier. In some examples, 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.

[0019a] In a second aspect, there is provided a pharmaceutical composition comprising: (a) the anti-4-1BB antibody or antigen-binding fragment as disclosed herein; and (b) a pharmaceutically acceptable carrier.

[0020] 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 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.

[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.

[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.

[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

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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, an immune checkpoint inhibitor is an agent that inhibits PD-1 signaling. In some examples, an agent that inhibits PD-1 signaling is an anti-PD-1 antibody. In some examples, an anti-PD-1 antibody is nivolumab, pembrolizumab, atezolizumab, durvalumab, or avelumab.

[0029] In some examples, 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 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.

[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.

[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.

[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

the coated surface; and (d) removing unattached cells, thereby isolating antigen-specific activated T cells. In some examples, activated T cells are CD8⁺ T cells.

[0033] In some examples, 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 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 some examples, a composition includes least 10⁹, at least 10¹⁰ cells, or more than 10¹⁰ activated T cells. In some examples, activated T cells are CD8⁺ T cells.

[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 examples, 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 examples, 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.

[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 disclosure are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating examples of the present disclosure, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the disclosure 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, an IgG2, an IgG4, an IgA, an IgE, an IgM, and an IgD.
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, or a scFv fragment.
15. A pharmaceutical composition comprising:
 - (a) the anti-4-1BB antibody or antigen-binding fragment of any one of claims 1-14; and
 - (b) a pharmaceutically acceptable carrier.