

What claimed is:

1. A bispecific antibody comprising a first antigen-binding functional region that specifically binds to PD-L1 and a second antigen-binding functional region that specifically binds to 4-1BB, wherein

5 the first antigen-binding functional region that specifically binds PD-L1 comprises:

(A) a heavy chain variable region, comprising

(a) HCDR1, which comprises the amino acid sequence set forth in SEQ ID NO: 15,

(b) HCDR2, which comprises the amino acid sequence set forth in SEQ ID NO: 16, and

(c) HCDR3, which comprises the amino acid sequence set forth in SEQ ID NO: 17; and

10 (B) a light chain variable region, comprising

(a) LCDR1, which comprises the amino acid sequence set forth in SEQ ID NO: 18,

(b) LCDR2, which comprises the amino acid sequence set forth in SEQ ID NO: 19, and

(c) LCDR3, which comprises the amino acid sequence set forth in SEQ ID NO: 20.

2. The bispecific antibody of claim 1, wherein

15 the second antigen-binding functional region that specifically binds to 4-1BB comprises

(A) a heavy chain variable region, comprising

(a) HCDR1, which comprises the amino acid sequence set forth in SEQ ID NO: 21,

(b) HCDR2, which comprises the amino acid sequence set forth in SEQ ID NO: 22, and

(c) HCDR3, which comprises the amino acid sequence set forth in SEQ ID NO: 23; and

20 (B) a light chain variable region, comprising

(a) LCDR1, which comprises the amino acid sequence set forth in SEQ ID NO: 24,

(b) LCDR2, which comprises the amino acid sequence set forth in SEQ ID NO: 25, and

(c) LCDR3, which comprises the amino acid sequence set forth in SEQ ID NO: 26.

3. The bispecific antibody of claim 1 or claim 2, wherein

25 the first antigen-binding functional region that specifically binds to PD-L1 comprises

(A) a heavy chain variable region, comprising

(a) HCDR1, which comprises the amino acid sequence set forth in SEQ ID NO: 15,

(b) HCDR2, which comprises the amino acid sequence set forth in SEQ ID NO: 16, and

(c) HCDR3, which comprises the amino acid sequence set forth in SEQ ID NO: 17; and

30 (B) a light chain variable region, comprising

(a) LCDR1, which comprises the amino acid sequence set forth in SEQ ID NO: 18,
(b) LCDR2, which comprises the amino acid sequence set forth in SEQ ID NO: 19, and
(c) LCDR3, which comprises the amino acid sequence set forth in SEQ ID NO: 20; and
the second antigen-binding functional region that specifically binds to 4-1BB comprises

5 (A) a heavy chain variable region, comprising

(a) HCDR1, which comprises the amino acid sequence set forth in SEQ ID NO: 21,
(b) HCDR2, which comprises the amino acid sequence set forth in SEQ ID NO: 22, and
(c) HCDR3, which comprises the amino acid sequence set forth in SEQ ID NO: 23; and

(B) a light chain variable region, comprising

10 (a) LCDR1, which comprises the amino acid sequence set forth in SEQ ID NO: 24,
(b) LCDR2, which comprises the amino acid sequence set forth in SEQ ID NO: 25, and
(c) LCDR3, which comprises the amino acid sequence set forth in SEQ ID NO: 26.

4. The bispecific antibody of any one of claims 1-3, wherein the first antigen-binding functional
region that specifically binds to PD-L1 comprises a heavy chain variable region comprising the
15 amino acid sequence set forth in SEQ ID NO: 6, and a light chain variable region comprising the
amino acid sequence set forth in SEQ ID NO: 2.

5. The bispecific antibody of any one of claims 1-4, wherein the second antigen-binding
functional region that specifically binds to 4-1BB comprises a heavy chain variable region
comprising the amino acid sequence set forth in SEQ ID NO: 12, and a light chain variable region
20 comprising the amino acid sequence set forth in SEQ ID NO: 10.

6. The bispecific antibody of any one of claims 1-5,

wherein the first antigen-binding functional region that specifically binds to PD-L1
comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID
NO: 6, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID
25 NO: 2; and

wherein the second antigen-binding functional region that specifically binds to 4-1BB
comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID
NO: 12, and a light chain variable region comprising the amino acid sequence set forth in SEQ
ID NO: 10.

30 7. The bispecific antibody of any one of claims 1-6, wherein the first antigen-binding functional

region and the second antigen-binding functional region are selected from the group consisting of Fab fragments, scFv fragments, and variable domain fragments Fv.

8. The bispecific antibody of any one of claims 1-7, wherein the first antigen-binding functional region and the second antigen-binding functional region are both Fab fragments.

5 9. The bispecific antibody of any one of claims 1-8, wherein the Fab fragment thereof comprises a different first heavy chain variable region and a different second heavy chain variable region, as well as a different first light chain variable region and a different second light chain variable region.

10 10. The bispecific antibody of any one of claims 1-9, wherein one of the first antigen-binding functional region and the second antigen-binding functional region is a Fab fragment, and the other is a scFv.

11. The bispecific antibody of any one of claims 1-10, comprising a first Fc chain and a second Fc chain,

15 wherein the first Fc chain and the second Fc chain are both immunoglobulin G Fc fragments containing amino acid substitutions, and the first Fc chain and the second Fc chain together constitute a heterodimer that can bind to the Fc receptor;

wherein the first Fc chain and the second Fc chain are linked to the first antigen-binding functional region and the second antigen-binding functional region, respectively, via a covalent bond or a linker; and

20 wherein either the first Fc chain or the second Fc chain comprises T366L and D399R amino acid substitutions at positions 366 and 399, and the other comprises L351E, Y407L and K409V amino acid substitutions at positions 351, 407 and 409, wherein the amino acid positions are numbered according to the Kabat EU index numbering system.

25 12. The bispecific antibody of any one of claims 1-11, wherein the weight ratios of the homodimers formed by the first Fc chain and the first antigen-binding functional region covalently linked thereto and by the second Fc chain and the second antigen-binding functional region covalently linked thereto of said bispecific antibody are less than 50% of the total amount of all the polypeptide chains, in a reducing agent-containing solution in which no other polypeptides present except for said Fc chains and the antigen-binding functional regions.

30 13. The bispecific antibody of any one of claims 1-12, comprising

a first heavy chain/first light chain pair that specifically binds to PD-L1, wherein the first heavy chain has a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 6, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 8; the first light chain has a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 4.

14. The bispecific antibody of any one of claims 1-13, comprising

a second heavy chain/second light chain pair that specifically binds to 4-1BB, wherein the second heavy chain has a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 12, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 14; the second light chain has a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 10, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 4.

15. An isolated polynucleotide encoding the bispecific antibody of any one of claims 1-14.

16. A recombinant expression vector comprising the isolated polynucleotide of claim 15.

17. A host cell comprising the isolated polynucleotide of claim 15 or the recombinant expression vector of claim 16.

18. The host cell of claim 17, selected from the group consisting of human embryonic kidney cells HEK293, or HEK293T, HEK293E, HEK293F that are modified from HEK293 cells; hamster ovary cells CHO, or CHO-S, CHO-dhfr-, CHO/DG44, ExpiCHO that are modified from CHO cells; *Escherichia coli*, or *Escherichia coli* BL21, BL21(DE3), Rosetta, Origami that are modified from *E. coli*; Yeast, or *Pichia*, *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Hansenula polymorpha* that are modified from Yeast; insect cells, or High5, SF9 cells that are modified from insect cells; plant cells; mammalian mammary gland cells; somatic cells.

19. A composition comprising the bispecific antibody of any one of claims 1-14 or the isolated polynucleotide of claim 15 or the recombinant expression vector of claim 16 or the host cell of claim 17 or 18, and a pharmaceutically acceptable carrier.

20. The composition of claim 19, further comprising a second therapeutic agent, preferably, the second therapeutic agent and the bispecific antibody, the isolated polynucleotide, the recombinant expression vector or the host cell are located in different parts of the composition, and preferably,

the second therapeutic agent is selected from the group consisting of a second antibody, an immunotherapeutic agent, a targeted therapeutic agent or a chemotherapeutic agent, preferably, the second therapeutic agent is an anti-PD-1 antibody and/or a STING agonist.

21. A method of producing the bispecific antibody of any one of claims 1-14, including the steps of:

- 1) Expressing the isolated polynucleotide of claim 15 or the recombinant expression vector of claim 16 in host cells, respectively;
- 2) Reducing the proteins respectively expressed in the host cells; and
- 3) Mixing the reduced proteins, and then oxidizing the mixture.

22. The method of claim 21, wherein the reduction step includes 1) performing the reduction reaction in the presence of a reducing agent selected from the group consisting of 2-mercaptoethylamine, dithiothreitol, tris(2-carboxyethyl) phosphine or other chemical derivatives thereof; 2) Removing the reducing agent.

23. The method of claim 21 or 22, wherein the oxidation step is conducted in the air or in the presence of an oxidizing agent selected from the group consisting of L-dehydroascorbic acid or chemical derivatives thereof.

24. The method of any one of claims 21-23, further including a step of separation and purification.

25. Use of the bispecific antibody of any one of claims 1-14 and/or the isolated polynucleotide of claim 15 and/or the recombinant expression vector of claim 16 and/or the host cell of claim 17 or 18 and/or the composition of claim 19 or 20, in the manufacture of a medicament for preventing and/or treating diseases in a subject, wherein the diseases are selected from the group consisting of the following tumors: leukemia, lymphoma, myeloma, brain tumor, head and neck squamous cell cancer, non-small cell lung cancer, nasopharyngeal cancer, esophageal cancer, gastric cancer, pancreatic cancer, gallbladder cancer, liver cancer, colorectal cancer, breast cancer, ovarian cancer, cervical cancer, endometrial cancer, uterine sarcoma, prostate cancer, bladder cancer, renal cell cancer, melanoma, small cell lung cancer and bone cancer.

26. The bispecific antibody of any one of claims 1-14 and/or the isolated polynucleotide of claim 15 and/or the recombinant expression vector of claim 16 and/or the host cell of claim 17 or 18 and/or the composition of claim 19 or 20, for use as a medicament ~~for preventing and/or treating diseases in a subject.~~

27. ~~A method of preventing and/or treating diseases~~Use of claim 25, including wherein the
medicament is to be administered ~~administering~~ to a subject in need thereof the bispecific
antibody ~~in a heterodimeric form~~ of any one of claims 1-14 and/or the isolated bispecific antibody
of claim 15 and/or the recombinant expression vector of claim 16 and/or the host cell of claim 17
5 or 18 and/or the composition of claim 19 or 20.

28. Use of claim 25, the bispecific antibody ~~in a heterodimeric form~~, the isolated polynucleotide,
the recombinant expression vector, the host cell or the composition of claim 26, or the ~~method~~
use of claim 27, wherein the subject is a mammal, preferably a human subject.

29. ~~Use of claim 25, the~~The bispecific antibody in a heterodimeric form, the isolated polynucleotide,
10 the recombinant expression vector, the host cell or the composition of claim 26, ~~or the method of~~
~~claim 27, wherein the~~ for use in the treatment of diseases in a subject, wherein the diseases are
selected from the group consisting of the following tumors: leukemia, lymphoma, myeloma, brain
tumor, head and neck squamous cell cancer, non-small cell lung cancer, nasopharyngeal cancer,
esophageal cancer, gastric cancer, pancreatic cancer, gallbladder cancer, liver cancer, colorectal
15 cancer, breast cancer, ovarian cancer, cervical cancer, endometrial cancer, uterine sarcoma,
prostate cancer, bladder cancer, renal cell cancer, melanoma, small cell lung cancer and bone
cancer.