CLAIMS

1. A bispecific antibody comprising a B7-H4-targeting antigen-binding domain and a 4-1BB-targeting antigen-binding domain, wherein the B7-H4-targeting antigen-binding domain comprises:

(1). HCDR1 with a sequence as set forth in SEQ ID NO: 16, HCDR2 with a sequence as set forth in SEQ ID NO: 60, HCDR3 with a sequence as set forth in SEQ ID NO: 84, LCDR1 with a sequence as set forth in SEQ ID NO: 112, LCDR2 with a sequence as set forth in SEQ ID NO: 118 and LCDR3 with a sequence as set forth in SEQ ID NO: 133; or

(2). HCDR1 with a sequence as set forth in SEQ ID NO: 16, HCDR2 with a sequence as set forth in SEQ ID NO: 46, HCDR3 with a sequence as set forth in SEQ ID NO: 84, LCDR1 with a sequence as set forth in SEQ ID NO: 112, LCDR2 with a sequence as set forth in SEQ ID NO: 118 and LCDR3 with a sequence as set forth in SEQ ID NO: 131.

2. The bispecific antibody according to claim 1, wherein the B7-H4-targeting antigen-binding domain comprises a VH with a sequence as set forth in SEQ ID NO: 160 and a VL with a sequence as set forth in SEQ ID NO: 168; or a VH with a sequence as set forth in SEQ ID NO: 142 and a VL with a sequence as set forth in SEQ ID NO: 166.

3. The bispecific antibody according to claim 1 or 2, wherein the B7-H4-targeting antigen-binding domain comprises a heavy chain with a sequence as set forth in SEQ ID NO: 192 and a light chain with a sequence as set forth in SEQ ID NO: 200; or a heavy chain with a sequence as set forth in SEQ ID NO: 174 and a light chain with a sequence as set forth in SEQ ID NO: 198.

4. The bispecific antibody according to claim 1, wherein the B7-H4-targeting antigen-binding domain is in the form of ScFv, Fab or IgG.

5. The bispecific antibody according to claim 4, wherein the B7-H4-targeting antigen-binding domain is in the form of the IgG, the B7-H4-targeting antigen-binding domain comprises a constant region derived from a human IgG1 comprising mutations L234A, L235A and P329G or mutations L234A and L235A.

6. The bispecific antibody according to any one of claims 1–5, wherein the 4-1BB-targeting antigen-binding domain comprises:

(1). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 61 and 95, respectively;

or, (2). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 17, 47 and 85, respectively;

or, (3). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 48 and 86, respectively;

or, (4). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 49 and 86, respectively;

or, (5). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 19, 49 and 94, respectively;

or, (6). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 49 and 87, respectively;

or, (7). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 19, 50 and 88, respectively;

or, (8). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 20, 51 and 89, respectively;

or, (9). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 52 and 90, respectively;

or, (10). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 49 and 90, respectively;

or, (11). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 21, 53 and 91, respectively;

or, (12). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 21, 54 and 92, respectively;

or, (13). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 19, 55 and 93, respectively;

or, (14). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 22, 56 and 86, respectively;

or, (15). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID

NOs: 18, 57 and 95, respectively;

or, (16). HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 19, 58 and 96, respectively.

7. The bispecific antibody according to any one of claims 1-6, wherein the 4-1BB-targeting antigen-binding domain comprises one or more heavy chain variable regions with a sequence as set forth in SEQ ID NO: 161, 143–144, 153-154, 145-152, 155-157, or 284.

8. The bispecific antibody according to any one of claims 1-7, wherein the 4-1BB-targeting antigen-binding domain comprises a heavy chain with a sequence as set forth in SEQ ID NO: 193, 175–176, 185-186, 177-184, 187-189, or 285.

9. The bispecific antibody according to any one of claims 1-8, wherein the 4-1BB-targeting antigen-binding domain is in the form of single VH or tandem VH, or HCAb.

10. The bispecific antibody according to any one of claims 1–9, wherein

the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 133, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 60 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 61 and 95, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 133, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 60 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 17, 47 and 85, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 133, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 60 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 48 and 86, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 133, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 60 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 49 and 86, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 133, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 60 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 19, 49 and 94, respectively;

the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 17, 47 and 85, respectively;

the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 48 and 86, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 49 and 87, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and

LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 19, 50 and 88, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 20, 51 and 89, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 52 and 90, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 21, 53 and 91, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 21, 54 and 92, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively,

and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 19, 55 and 93, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 49 and 86, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 19, 49 and 94, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 22, 56 and 86, respectively;

or, the B7-H4-targeting antigen-binding domain comprises LCDR1, LCDR2 and LCDR3 with sequences as set forth in SEQ ID NOs: 112, 118 and 131, respectively, and HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 16, 46 and 84, respectively; and the 4-1BB-targeting antigen-binding domain comprises HCDR1, HCDR2 and HCDR3 with sequences as set forth in SEQ ID NOs: 18, 57 and 95, respectively.

11. The bispecific antibody according to any one of claims 1–10, wherein the B7-H4-targeting antigen domain is in the form of IgG and the 4-1BB-targeting antigen-binding domain is in the form of single VH;

or, the B7-H4-targeting antigen domain is in the form of IgG and the 4-1BB-targeting antigen-binding domain is in the form of a tandem of 2 or 3 VHs;

or, the B7-H4-targeting antigen domain is in the form of Fab and the 4-1BB-targeting antigen-binding domain is in the form of HCAb or in the form of HCAb-VH;

or, the B7-H4-targeting antigen domain is in the form of Fab and the 4-1BB-targeting antigen-binding domain is in the form of VH.

12. The bispecific antibody according to any one of claims 1-10, wherein the bispecific antibody is in a form where:

(1) the bispecific antibody comprises a polypeptide chain 1 shown as a formula of VLB7-H4-CL, and a polypeptide chain 2 shown as a formula of VHB7-H4-CH1-hinge-CH2-CH3-linker-(VH4-1BB)n;

(2) the bispecific antibody comprises a polypeptide chain 1 shown as a formula of VHB7-H4-CH1, and a polypeptide chain 2 shown as a formula of VLB7-H4-CL-linker-VH4-1BB-linker-CH2-CH3;

(3) the bispecific antibody comprises a polypeptide chain 1 shown as a formula of VHB7-H4-CH1, and a polypeptide chain 2 shown as a formula of VLB7-H4-CL-linker-VH4-1BB-linker-CH2-CH3-VH4-1BB;

or (4) the bispecific antibody in this form comprises three polypeptide chains:

a polypeptide chain 1 shown as a formula of VLB7-H4-CL, a polypeptide chain 2 shown as a formula of VHB7-H4-CH1-hinge-CH2-CH3, and a polypeptide chain 3 shown as a formula of VH4-1BB-linker-CH2-CH3 or (VH4-1BB)n-linker-CH2-CH3.

13. The bispecific antibody according to claim 12, wherein the linker is selected from the group consisting of SEQ ID NOs: 241–261, 282 and 288–289.

14. The bispecific antibody according to claim 12 or 13, wherein

the linker in form (1) comprises a sequence as set forth in SEQ ID NO: 245;

the linker in form (2) comprises a sequence as set forth in SEQ ID NO: 250;

the linker in form (3) comprises a sequence as set forth in SEQ ID NO: 282; and

the linker in form (4) comprises a sequence as set forth in SEQ ID NO: 245.

15. The bispecific antibody according to claim 1, wherein

the bispecific antibody comprises a polypeptide chain 1 as set forth in SEQ ID NO: 200, and a polypeptide chain 2 as set forth in any one of SEQ ID NOs: 237, 235-

236, 218–225, 263–268, 274–281, 286 and 287;

the bispecific antibody comprises a polypeptide chain 1 as set forth in SEQ ID NO: 198, and a polypeptide chain 2 as set forth in any one of SEQ ID NOs: 203–215;

or, the bispecific antibody comprises a polypeptide chain 1 is as set forth in SEQ ID NO: 233, and a polypeptide chain 2 is as set forth in any one of SEQ ID NOs: 234 and 269–273.

16. An isolated nucleic acid encoding the bispecific antibody according to any one of claims 1–15.

17. An expression vector comprising the isolated nucleic acid according to claim 16.

18. A host cell comprising the expression vector according to claim 17, preferably, wherein the host cell is a prokaryotic cell or a eukaryotic cell.

19. A pharmaceutical composition comprising the bispecific antibody according to any one of claims 1–15.

20. A chimeric antigen receptor comprising the bispecific antibody according to any one of claims 1–15.

21. An antibody-drug conjugate comprising a cytotoxic agent and the bispecific antibody according to any one of claims 1–15, wherein preferably, the cytotoxic agent is MMAF or MMAE.

22. A combination of kits comprising a kit A and a kit B, wherein:

the kit A comprises the bispecific antibody according to any one of claims 1–15, the antibody-drug conjugate according to claim 21 and/or the pharmaceutical composition according to claim 19;

the kit B comprises another anti-tumor antibody or a pharmaceutical composition comprising the anti-tumor antibody, and/or one or more of the group consisting of a hormonal agent, a small molecule-targeted agent, a proteasome inhibitor, an imaging agent, a diagnostic agent, a chemotherapeutic agent, an oncolytic drug, a cytotoxic agent, a cytokine, an activator of a co-stimulatory molecule, an inhibitor of an inhibitory molecule, and a vaccine.

23. Use of a therapeutically effective amount of the bispecific antibody according to any one of claims 1–15, the antibody-drug conjugate according to claim 21 or the

pharmaceutical composition according to claim 19, or the combination of kits according to claim 22 in the manufacture of a medicament for treating and/or preventing a 4-1BB and/or B7-H4-mediated tumor in a patient in need thereof.

24. The use according to claim 23, wherein the tumor is breast cancer, ovarian cancer, endometrial cancer, renal cancer, melanoma, lung cancer, gastric cancer, liver cancer, esophageal cancer, cervical cancer, head and neck tumor, cholangiocarcinoma, gallbladder cancer, bladder cancer, sarcoma or colorectal cancer; preferably, the cancer is breast cancer, ovarian cancer, endometrial cancer, renal cancer or cholangiocarcinoma; more preferably, the cancer is breast cancer.