

CLAIMS

1. An isolated bispecific antibody or antigen-binding fragment thereof comprising:

- a. a first heavy chain, H1;
- b. a second heavy chain, H2;
- c. a first light chain, L1; and
- d. a second light chain, L2;

wherein H1 and L1 form a first arm comprising a first antigen-binding domain that specifically binds a first antigen, preferably a first antigen of human origin, and wherein H2 and L2 form a second arm comprising a second antigen-binding domain that specifically binds a second antigen, preferably a second antigen of human origin, wherein

- (a) H1 and H2 each comprises a CH1 region of human IgG1, IgG2, IgG3, or IgG4; and
- (b) L1 and L2 each comprises a CL region of a human kappa light chain or a human lambda light chain;

wherein H1L1 and H2L2 each comprise a charge pair selected from the group consisting of the following amino acid substitutions:

- (1) G166D/E in CH1 of H1 and S114K/R in CL of L1, respectively, and G166K/R in CH1 of H2 and S114D/E in CL of L2, respectively;
- (2) T187D/E in CH1 of H1 and D/N170K/R in CL of L1, respectively, and T187K/R in CH1 of H2 and D/N170D/E in CL of L2, respectively;
- (3) S131D/E in CH1 of H1 and P119K/R in CL of L1, respectively, and S131K/R in CH1 of H2 and P119D/E in CL of L2, respectively;
- (4) A129D/E in CH1 of H1 and S121K/R in CL of L1, respectively, and A129K/R in CH1 of H2 and S121D/E in CL of L2, respectively;
- (5) G166D/E in CH1 of H2 and S114K/R in CL of L2, respectively, and G166K/R in CH1 of H1 and S114D/E in CL of L1, respectively;
- (6) T187D/E in CH1 of H2 and D/N170K/R in CL of L2, respectively, and T187K/R in CH1 of H1 and D/N170D/E in CL of L1, respectively;
- (7) S131D/E in CH1 of H2 and P119K/R in CL of L2, respectively, and S131K/R in CH1 of H1 and P119D/E in CL of L1, respectively; or
- (8) A129D/E in CH1 of H2 and S121K/R in CL of L2, respectively, and A129K/R in CH1 of H1 and S121D/E in CL of L1, respectively.

2. The isolated bispecific antibody or antigen-binding fragment thereof of claim 1, wherein

- (a) the two heavy chains H1 and H2 each comprise a VH region, a CH1 region, and a Fc region (containing CH2 and CH3 regions), wherein the VH regions have different amino acid sequences;
 - (b) the two heavy chains H1 and H2 each comprise a VH region, a CH1 region, and a Fc region (containing CH2 and CH3 regions), wherein the CH1 regions have different amino acid sequences;
 - (c) the two heavy chains H1 and H2 each comprise a VH region, a CH1 region, and a Fc region (containing CH2 and CH3 regions), wherein the Fc regions have different amino acid sequences;
 - (d) the two light chains L1 and L2 each comprise a VL region and a CL region, wherein the VL regions have different amino acid sequences; and/or
 - (e) the two light chains L1 and L2 each comprise a VL region and a CL region, wherein the CL regions have different amino acid sequences.
3. The isolated bispecific antibody or antigen-binding fragment thereof of claim 2, wherein H1 and H2 form a heterodimer.
4. The isolated bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 3, wherein the isolated bispecific antibody comprises:
- (a) a negatively charged amino acid (D or E) at G166, T187, S131, or A129 in CH1 of H1, the VH region of H1 and the VL region of L1 have a Q39E and a Q38K substitution mutation, respectively, and the VH region of H2 and the VL region of L2 have a Q39K and a Q38E substitution mutation, respectively; or
 - (b) a positively charged amino acid (K or R) at G166, T187, S131, or A129 in CH1 of H1, the VH region of H1 and the VL region of L1 have a Q39K and a Q38E substitution mutation, respectively, and the VH region of H2 and the VL region of L2 have a Q39E and a Q38K substitution mutation, respectively.
5. The isolated bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 4,
- wherein the CH1 and CL regions of one of the two arms comprise amino acid substitutions at an amino acid residue corresponding to the amino acid position of SEQ ID NO:17, 18, 19, or 20 for CH1 and SEQ ID NO:21 or 22 for CL;
- wherein the amino acid substitutions in the CH1 and CL regions are selected from:

- (1) K133C and C220X in CH1, and F209C and C214X in CL;
- (2) R133C and C131X in CH1, and F209C and C214X in CL;
- (3) R133C and C131X in CH1, and V209C and C214X in CL; or
- (4) K133C and C220X in CH1, and V209C and C214X in CL;

wherein X is selected from S, A or G

6. The isolated bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 5, wherein the first antigen-binding domain specifically binds CD47, preferably human CD47, and the second antigen-binding domain specifically binds a TAA expressed on the same cell as CD47, preferably human TAA.

7. The isolated bispecific antibody or antigen-binding fragment thereof of claim 6, wherein the second antigen-binding domain specifically binds CLDN18.2 expressed on the same cell as CD47, preferably human CLDN18.2.

8. The isolated bispecific antibody or antigen-binding fragment thereof of claim 6 or 7, wherein the anti-CD47 antigen-binding domain comprises the VH, CH1, VL, and CL comprising the amino acid sequences of:

- (1) SEQ ID NOs: 1, 27, 2 and 29, respectively;
- (2) SEQ ID NOs: 1, 28, 2 and 29, respectively;
- (3) SEQ ID NOs: 1, 27, 2 and 30, respectively;
- (4) SEQ ID NOs: 1, 28, 2 and 30, respectively;
- (5) SEQ ID NOs: 1, 31, 2 and 33, respectively;
- (6) SEQ ID NOs: 1, 32, 2 and 33, respectively;
- (7) SEQ ID NOs: 1, 31, 2 and 34, respectively;
- (8) SEQ ID NOs: 1, 32, 2 and 34, respectively;
- (9) SEQ ID NOs: 1, 35, 2 and 37, respectively;
- (10) SEQ ID NOs: 1, 36, 2 and 37, respectively;
- (11) SEQ ID NOs: 1, 35, 2 and 38, respectively;
- (12) SEQ ID NOs: 1, 36, 2 and 38, respectively;
- (13) SEQ ID NOs: 1, 39, 2 and 41, respectively;
- (14) SEQ ID NOs: 1, 40, 2 and 41, respectively;
- (15) SEQ ID NOs: 1, 39, 2 and 42, respectively;
- (16) SEQ ID NOs: 1, 40, 2 and 42, respectively;

- (17) SEQ ID NOs: 1, 43, 2 and 45, respectively;
- (18) SEQ ID NOs: 1, 44, 2 and 45, respectively;
- (19) SEQ ID NOs: 1, 43, 2 and 46 respectively;
- (20) SEQ ID NOs: 1, 44, 2 and 46, respectively;
- (21) SEQ ID NOs: 1, 47, 2 and 49, respectively;
- (22) SEQ ID NOs: 1, 48, 2 and 49, respectively;
- (23) SEQ ID NOs: 1, 47, 2 and 50, respectively;
- (24) SEQ ID NOs: 1, 48, 2 and 50, respectively;
- (25) SEQ ID NOs: 1, 51, 2 and 53, respectively;
- (26) SEQ ID NOs: 1, 52, 2 and 53, respectively;
- (27) SEQ ID NOs: 1, 51, 2 and 54, respectively;
- (28) SEQ ID NOs: 1, 52, 2 and 54, respectively;
- (29) SEQ ID NOs: 1, 55, 2 and 57, respectively;
- (30) SEQ ID NOs: 1, 56, 2 and 57, respectively;
- (31) SEQ ID NOs: 1, 55, 2 and 58, respectively;
- (32) SEQ ID NOs: 1, 56, 2 and 58, respectively;
- (33) SEQ ID NOs: 23, 27, 24 and 29, respectively;
- (34) SEQ ID NOs: 23, 28, 24 and 29, respectively;
- (35) SEQ ID NOs: 23, 27, 24 and 30, respectively;
- (36) SEQ ID NOs: 23, 28, 24 and 30, respectively;
- (37) SEQ ID NOs: 25, 31, 26 and 33, respectively;
- (38) SEQ ID NOs: 25, 32, 26 and 33, respectively;
- (39) SEQ ID NOs: 25, 31, 26 and 34, respectively;
- (40) SEQ ID NOs: 25, 32, 26 and 34, respectively;
- (41) SEQ ID NOs: 23, 35, 24 and 37, respectively;
- (42) SEQ ID NOs: 23, 36, 24 and 37, respectively;
- (43) SEQ ID NOs: 23, 35, 24 and 38, respectively;
- (44) SEQ ID NOs: 23, 36, 24 and 38, respectively;
- (45) SEQ ID NOs: 25, 39, 26 and 41, respectively;
- (46) SEQ ID NOs: 25, 40, 26 and 41, respectively;
- (47) SEQ ID NOs: 25, 39, 26 and 42, respectively;
- (48) SEQ ID NOs: 25, 40, 26 and 42, respectively;
- (49) SEQ ID NOs: 23, 43, 24 and 45, respectively;
- (50) SEQ ID NOs: 23, 44, 24 and 45, respectively;

- (51) SEQ ID NOs: 23, 43, 24 and 46 respectively;
- (52) SEQ ID NOs: 23, 44, 24 and 46, respectively;
- (53) SEQ ID NOs: 25, 47, 26 and 49, respectively;
- (54) SEQ ID NOs: 25, 48, 26 and 49, respectively;
- (55) SEQ ID NOs: 25, 47, 26 and 50, respectively;
- (56) SEQ ID NOs: 25, 48, 26 and 50, respectively;
- (57) SEQ ID NOs: 23, 51, 24 and 53, respectively;
- (58) SEQ ID NOs: 23, 52, 24 and 53, respectively;
- (59) SEQ ID NOs: 23, 51, 24 and 54, respectively;
- (60) SEQ ID NOs: 23, 52, 24 and 54, respectively;
- (61) SEQ ID NOs: 25, 55, 26 and 57, respectively;
- (62) SEQ ID NOs: 25, 56, 26 and 57, respectively;
- (63) SEQ ID NOs: 25, 55, 26 and 58, respectively; or
- (64) SEQ ID NOs: 25, 56, 26 and 58, respectively.

9. The isolated bispecific antibody or antigen-binding fragment thereof of claim 8, wherein the first antigen-binding domain comprises the VH, CH1, VL, and CL, the second antigen-binding domain comprises the VH, CH1, VL, and CL, comprising the amino acid sequences of:

- (1) SEQ ID: 1, 28, 2, 29, 3, 63, 4 and 64, respectively;
- (2) SEQ ID: 1, 36, 2, 37, 3, 67, 4 and 68, respectively;
- (3) SEQ ID: 1, 44, 2, 45, 3, 71, 4 and 72, respectively;
- (4) SEQ ID: 1, 52, 2, 53, 3, 73, 4 and 74, respectively;
- (5) SEQ ID: 1, 31, 2, 34, 3, 65, 4 and 66, respectively;
- (6) SEQ ID: 1, 39, 2, 42, 3, 69, 4 and 70, respectively;
- (7) SEQ ID: 1, 47, 2, 50, 3, 75, 4 and 76, respectively;
- (8) SEQ ID: 1, 55, 2, 58, 3, 77, 4 and 78, respectively;
- (9) SEQ ID: 23, 28, 24, 29, 59, 63, 60 and 64, respectively;
- (10) SEQ ID: 23, 36, 24, 37, 59, 67, 60 and 68, respectively;
- (11) SEQ ID: 23, 44, 24, 45, 59, 71, 60 and 72, respectively;
- (12) SEQ ID: 23, 52, 24, 53, 59, 73, 60 and 74, respectively;
- (13) SEQ ID: 25, 31, 26, 34, 61, 65, 62 and 66, respectively;
- (14) SEQ ID: 25, 39, 26, 42, 61, 69, 62 and 70, respectively;
- (15) SEQ ID: 25, 47, 26, 50, 61, 75, 62 and 76, respectively; or

(16) SEQ ID: 25, 55, 26, 58, 61, 77, 62 and 78, respectively.

10. The isolated bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 5, wherein the isolated bispecific antibody or antigen-binding fragment thereof comprises an anti-immune cell modulator (ICM) antibody or antigen-binding fragment arm thereof and is capable of specific binding to ICM.

11. The isolated bispecific antibody or antigen-binding fragment thereof of claim 10, wherein the ICM is selected from the group consisting of CD3, CD16, CD27, CD28, CD40, CD122, NKp46, OX40, 4-1BB, GITR, ICOS, CTLA-4, PD-1, LAG-3, TIM-3, VISTA, SIGLEC7, SIGLEC9, KIR, BTLA, B7-H3, and other cell surface immune regulatory molecules.

12. The isolated bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 5 and 10 to 11, wherein the first antigen-binding domain specifically binds CD3, preferably human CD3, and comprises the VH, CH1, VL, and CL comprising the amino acid sequences of SEQ ID: 9, 10, 11 and 12, respectively.

13. The isolated bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 5 and 10 to 12, wherein the first antigen-binding domain specifically binds CD3, preferably human CD3, and the second antigen-binding domain specifically binds DLL3, preferably human DLL3.

14. The isolated bispecific antibody or antigen-binding fragment thereof of claim 13, wherein the first antigen-binding domain comprises the VH, CH1, VL, and CL comprising the amino acid sequences of SEQ ID:9, 10, 11 and 12, respectively, and the second antigen-binding domain comprises the VH, CH1, VL, and CL comprising the amino acid sequences of SEQ ID:13, 14, 15 and 16, respectively.

15. An isolated nucleic acid encoding the isolated bispecific antibodies or antigen-binding fragments thereof of any one of claims 1 to 14.

16. A vector comprising the isolated nucleic acid thereof of claim 15.

17. A host cell comprising the vector thereof of claim 16.

18. A pharmaceutical composition, comprising the isolated bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 14 and a pharmaceutically acceptable carrier.
19. The pharmaceutical composition of claim 18 for use in therapy, wherein the pharmaceutical composition is to be administered to a subject in need thereof.
20. Use of a pharmaceutical composition comprising the isolated bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 14 and a pharmaceutically acceptable carrier in the manufacture of a medicament for the treatment of cancer and/or for use in a method of targeting one or two antigens expressed on a cancer cell surface including using CD47 blockade induced activation of macrophage-mediated phagocytosis or CD3-mediated T cell activation, wherein the pharmaceutical composition is to be administered to a subject in need thereof, and wherein optionally the cancer is selected from the group consisting of a lung cancer, a gastric cancer, an esophageal cancer, a bile duct cancer, a cholangiocarcinoma, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.
21. A method of producing the bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 14, comprising culturing a cell comprising a nucleic acid encoding the bispecific antibody or antigen-binding fragment thereof under conditions to produce the bispecific antibody or antigen-binding fragment thereof, and recovering the bispecific antibody or antigen-binding fragment thereof from the cell or culture.
22. A method of producing a pharmaceutical composition comprising the bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 14, comprising combining the bispecific antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.