

- the first part of the second antigen binding domain capable of specific binding to a first target is fused either directly or via a first peptide linker to the N-terminus of the spacer domain,

- the second part of the second antigen binding domain capable of specific binding to a first target is fused either directly or via a second peptide linker to the C-terminus of the spacer domain, and

- the second part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the second antigen binding domain capable of specific binding to a first target or is fused either directly or via a third peptide linker to the N-terminus of the first part of the second antigen binding domain capable of specific binding to a first target,

wherein the first part and the second part of the antigen binding domain capable of specific binding to the second target are associated with each other to form the antigen binding domain capable of specific binding to the second target and wherein the first part and the second part of the first and second antigen binding domain capable of specific binding to the first target are associated with each other to form a circular fusion polypeptide, and

wherein the spacer domain of the first fusion polypeptide and the spacer domain of the second fusion polypeptide are associated covalently to each other by a disulfide bond and comprise modifications promoting the association of the first and second fusion polypeptide.

In one aspect, the present disclosure provides a bispecific antibody consisting of two fusion polypeptides and comprising two antigen binding domains capable of specific binding to a first target and one antigen binding domain capable of specific binding to a second target comprising

(a) a first fusion polypeptide comprising an antibody heavy chain Fab fragment (VH-CH1) capable of specific binding to the first target, a spacer domain, an antibody light chain Fab fragment (VL-Ckappa) capable of specific binding to the first target and an antibody cross-Fab fragment comprising VH-Ckappa capable of specific binding to the second target, wherein

- the spacer domain comprises an antibody hinge region or a fragment thereof and an IgG1 Fc domain,

- the antibody heavy chain Fab fragment (VH-CH1) capable of specific binding to the first target is fused via a first peptide linker to the N-terminus of the spacer domain,

- the antibody light chain Fab fragment (VL-Ckappa) capable of specific binding to the first target is fused via a second peptide linker to the C-terminus of the spacer domain, and

- the antibody cross-Fab fragment comprising VH-Ckappa capable of specific binding to a second target is fused via a third peptide linker to the C-terminus of the antibody light chain Fab fragment (VL-Ckappa) capable of specific binding to the first target or is fused via a third peptide linker to the N-terminus of the antibody heavy chain Fab fragment (VH-CH1) capable of specific binding to the first target, and

(b) a second fusion polypeptide comprising a second antibody heavy chain Fab fragment (VH-CH1) capable of specific binding to a first target, a spacer domain, a second antibody light chain Fab fragment (VL-Ckappa) capable of specific binding to a first target and the an antibody cross-Fab fragment comprising VL-CH1 capable of specific binding to a second target, wherein

- the spacer domain comprises an antibody hinge region or a fragment thereof and an IgG1 Fc domain,

- the second antibody heavy chain Fab fragment (VH-CH1) capable of specific binding to a first target is fused via a first peptide linker to the N-terminus of the spacer domain,

- the antibody light chain Fab fragment (VL-Ckappa) capable of specific binding to a first target is fused via a second peptide linker to the C-terminus of the spacer domain, and

- the antibody cross-Fab fragment comprising VL-CH1 capable of specific binding to a second target is fused via a third peptide linker to the C-terminus of the second antibody light chain Fab fragment (VL-Ckappa) capable of specific binding to a first target or is fused via a third peptide linker to the N-terminus of the second antibody heavy chain Fab fragment (VH-CH1) capable of specific binding to a first target,

and wherein the antibody cross-Fab fragment comprising VH-Ckappa and the antibody cross-Fab fragment comprising VL-CH1 capable of specific binding to the second target are associated with each other to form the antigen binding domain capable of specific binding to the second target and wherein the antibody heavy chain Fab fragment (VH-CH1) and the antibody light chain Fab fragment (VL-Ckappa) of the first and second antigen binding domain capable of specific binding to the first target are associated with each other to form a circular fusion polypeptide, and

wherein the spacer domain of the first fusion polypeptide and the spacer domain of the second fusion polypeptide are associated covalently to each other by a disulfide bond and comprise knob-into-hole modifications promoting the association of the first and second fusion polypeptide.

In one example, provided is a bispecific antibody as defined herein before, wherein in the first fusion polypeptide the first part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the first antigen binding domain capable of specific binding to the first target and wherein in the second fusion polypeptide the second part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the first antigen binding domain capable of specific binding to the first target.

In another example, provided is a bispecific antibody as defined herein before, wherein in the first fusion polypeptide the first part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the N-terminus of the first part of the first antigen binding domain capable of specific binding to the first target and wherein in the second fusion polypeptide the second part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the N-

Claims

1. A bispecific antibody consisting of two fusion polypeptides and comprising two antigen binding domains capable of specific binding to a first target and one antigen binding domain capable of specific binding to a second target comprising

- 5 (a) a first fusion polypeptide comprising an antibody heavy chain Fab fragment (VH-CH1) capable of specific binding to the first target, a spacer domain, an antibody light chain Fab fragment (VL-Ckappa) capable of specific binding to the first target and an antibody cross-Fab fragment comprising VH-Ckappa capable of specific binding to the second target, wherein
 - 10 - the spacer domain comprises an antibody hinge region or a fragment thereof and an IgG1 Fc domain,
 - the antibody heavy chain Fab fragment (VH-CH1) capable of specific binding to the first target is fused via a first peptide linker to the N-terminus of the spacer domain,
 - 15 - the antibody light chain Fab fragment (VL-Ckappa) capable of specific binding to the first target is fused via a second peptide linker to the C-terminus of the spacer domain, and
 - the antibody cross-Fab fragment comprising VH-Ckappa capable of specific binding to a second target is fused via a third peptide linker to the C-terminus of the
 - 20 antibody light chain Fab fragment (VL-Ckappa) capable of specific binding to the first target or is fused via a third peptide linker to the N-terminus of the antibody heavy chain Fab fragment (VH-CH1) capable of specific binding to the first target, and
- 25 (b) a second fusion polypeptide comprising a second antibody heavy chain Fab fragment (VH-CH1) capable of specific binding to a first target, a spacer domain, a second antibody light chain Fab fragment (VL-Ckappa) capable of specific binding to a first target and an antibody cross-Fab fragment comprising VL-CH1 capable of specific binding to a second target, wherein
 - the spacer domain comprises an antibody hinge region or a fragment thereof and an
 - 30 IgG1 Fc domain,
 - the second antibody heavy chain Fab fragment (VH-CH1) capable of specific binding to a first target is fused via a first peptide linker to the N-terminus of the spacer domain,

- the second antibody light chain Fab fragment (VL-Ckappa) capable of specific binding to a first target is fused via a second peptide linker to the C-terminus of the spacer domain, and

- the antibody cross-Fab fragment comprising VL-CH1 capable of specific binding to a second target is fused via a third peptide linker to the C-terminus of the second antibody light chain Fab fragment (VL-Ckappa) capable of specific binding to a first target or is fused via a third peptide linker to the N-terminus of the second antibody heavy chain Fab fragment (VH-CH1) capable of specific binding to a first target,

and wherein the antibody cross-Fab fragment comprising VH-Ckappa and the antibody cross-Fab fragment comprising VL-CH1 capable of specific binding to the second target are associated with each other to form the antigen binding domain capable of specific binding to the second target and wherein the antibody heavy chain Fab fragment (VH-CH1) and the antibody light chain Fab fragment (VL-Ckappa) of the first and second antigen binding domain capable of specific binding to the first target are associated with each other to form a circular fusion polypeptide, and

wherein the spacer domain of the first fusion polypeptide and the spacer domain of the second fusion polypeptide are associated covalently to each other by a disulfide bond and comprise knob-into-hole modifications promoting the association of the first and second fusion polypeptide.

2. The bispecific antibody of claim 1, wherein the third peptide linker connecting the antibody cross-Fab fragment comprising VH-Ckappa or the antibody cross-Fab fragment comprising VL-CH1 capable of specific binding to a second target comprises at least 15 amino acids.
3. The bispecific antibody of claims 1 or 2, wherein the IgG1 Fc domain comprises amino acid substitutions L234A, L235A and P329G (numbering according to Kabat EU index).
4. The bispecific antibody of any one of claims 1 to 3, wherein the cross-Fab fragment capable of specific binding to a second target is an antigen binding domain capable of specific binding to a tumor associated antigen.
5. The bispecific antibody of any one of claims 1 to 4, wherein the cross-Fab fragment capable of specific binding to a second target is an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP).
6. The bispecific antibody of any one of claims 1 to 5, wherein the cross-Fab fragment capable of specific binding to a second target comprises
(a) a heavy chain variable region (V_HFAP) comprising (i) CDR-H1 comprising the amino

- acid sequence of SEQ ID NO:1, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:3, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:4, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:5, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:6, or
- (b) a heavy chain variable region (V_HFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:9, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:10, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:11, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:12, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:13, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:14.
7. The bispecific antibody of any one of claims 1 to 6, wherein the cross-Fab fragment capable of specific binding to a second target comprises
- (a) a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:7, and a light chain variable region (V_LFAP) comprising an amino acid sequence SEQ ID NO:8, or
- (b) a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:15, and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:16.
8. The bispecific antibody of any one of claims 1 to 7, wherein the Fab fragment capable of specific binding to a first target is an antigen binding domain capable of specific binding to OX40.
9. The bispecific antibody of any one of claims 1 to 8, wherein the Fab fragment capable of specific binding to a first target comprises
- (a) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:22, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:35, or
- (b) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:21, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:34, or

(c) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:23, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:36, or

(d) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:24, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:37, or

(e) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:25, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:29, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:32, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:38, or

(f) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:26, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:29, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:32, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:38, or

(g) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:27, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:30, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:33, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:39.

10. The bispecific antibody of any one of claims 1 to 9, wherein the Fab fragment capable of specific binding to a first target comprises

(a) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:40 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:41, or

(b) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:42 and a light chain variable region (V_LOX40) comprising an amino acid sequence of

SEQ ID NO:43, or

(c) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:44 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:45, or

5 (d) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:46 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:47, or

10 (e) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:48 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:49, or

(f) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:50 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:51, or

15 (g) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:52 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:53.

11. The bispecific antibody of any one of claims 1 to 10, wherein the Fab fragment capable of specific binding to a first target comprises

20 (a) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:40, and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:41.

12. The bispecific antibody of any one of claims 1 to 7, wherein the Fab fragment capable of specific binding to a first target is an antigen binding domain capable of specific binding to 4-1BB.

25 13. The bispecific antibody of any one of claims 1 to 7 or 12, wherein the Fab fragment capable of specific binding to a first target comprises a heavy chain variable region (V_H4-1BB) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:137, and a light chain variable region (V_L4-1BB) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:138, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:139, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:140.

30

14. The bispecific antibody of any one of claims 1 to 7 or 12 or 13, wherein the Fab fragment capable of specific binding to a first target comprises a heavy chain variable region (V_H4-

1BB) comprising an amino acid sequence of SEQ ID NO:141, and a light chain variable region (V_L4-1BB) comprising an amino acid sequence of SEQ ID NO:142.

15. The bispecific antibody of any one of claims 1 to 7, wherein the Fab fragment capable of specific binding to a first target is an antigen binding domain capable of specific binding to CD40.

16. The bispecific antibody of any one of claims 1 to 7 or 15, wherein the Fab fragment capable of specific binding to a first target comprises a heavy chain variable region (V_HCD40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:147, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:148, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:149, and a light chain variable region (V_LCD40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:150, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:151, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:152.

17. The bispecific antibody of any one of claims 1 to 7 or 15 or 16, wherein the Fab fragment capable of specific binding to a first target comprises a heavy chain variable region (V_HCD40) comprising an amino acid sequence of SEQ ID NO:153, and a light chain variable region (V_LCD40) comprising an amino acid sequence of SEQ ID NO:154.

18. The bispecific antibody of any one of claims 1 to 7 or 15 or 16, wherein the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to a first target comprises
(i) a heavy chain variable region (V_HCD40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:167, SEQ ID NO:168, SEQ ID NO:169 and SEQ ID NO:170, and a light chain variable region (V_LCD40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173, and SEQ ID NO:174, or
(ii) a heavy chain variable region (V_HCD40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, SEQ ID NO:178, SEQ ID NO:179 and SEQ ID NO:180, and a light chain variable region (V_LCD40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, and SEQ ID NO:184.

19. Isolated nucleic acid encoding the bispecific antibody of any one of claims 1 to 18.

20. A vector or host cell comprising the nucleic acid of claim 19.
21. A method of producing a bispecific antibody culturing the host cell of claim 20 under conditions suitable for the expression of the bispecific antibody.
22. A pharmaceutical composition comprising the bispecific antibody of any one of claims 8 to 18 and a pharmaceutically acceptable excipient.
23. The bispecific antibody of any one of claims 8 to 18 or the pharmaceutical composition of claim 22 for use as medicament.
24. The bispecific antibody of any one of claims 8 to 18 or the pharmaceutical composition of claim 22 for use in treating cancer or infectious diseases.
25. Use of the bispecific antibody of any one of claims 8 to 18 or the pharmaceutical composition of claim 22 in the manufacture of a medicament for the treatment of infections or cancer.
26. The pharmaceutical composition of claim 22, the bispecific antibody or the pharmaceutical composition for use of claim 23 or 24, or the use of claim 25, further comprising an additional therapeutic agent.
