

Claims

[Claim 1]

A method for producing a preparation of a multispecific antigen binding molecule, wherein the multispecific antigen binding molecule comprises:

(a) a first antigen-binding moiety and a second antigen-binding moiety, each of the first antigen-binding moiety and the second antigen-binding moiety is capable of binding to a first antigen and a second antigen different from the first antigen, but does not bind both antigens at the same time; and

(b) a third antigen-binding moiety capable of binding to a third antigen different from the first and the second antigen, preferably an antigen expressed on a cancer cell/tissue, wherein each of the first antigen-binding moiety and the second antigen-binding moiety comprises at least one cysteine residue (via mutation, substitution or insertion) which is not in a hinge region, preferably said at least one cysteine locates in the CH1 region; said at least one cysteine residue is capable of forming at least one disulfide bond between the first antigen-binding moiety and the second antigen-binding moiety, preferably in the CH1 region;

wherein said method comprises contacting the preparation with a reducing reagent.

[Claim 2]

The method of claim 1, wherein each of the first antigen-binding moiety and the second antigen-binding moiety comprises one cysteine residue (via mutation, substitution or insertion) at position 191 according to EU numbering in the CH1 region which is capable of forming one disulfide bond between the CH1 region of the first antigen-binding moiety and the CH1 region of the second antigen-binding moiety.

[Claim 3]

The method of claim 2, wherein said multispecific antigen binding molecule preparation (before contacting with the reducing agent) comprises two or more structural isoforms which differ by at least one disulfide bond formed between amino acid residues located in the CH1 region or at the position 191 in the CH1 region (EU numbering), and wherein the contacting with reducing agent preferentially enriches or increases the population of a structural isoform having at least one disulfide bond formed between amino acid residues located in the CH1 region or at the position 191 in the CH1 region (EU numbering).

- [Claim 4] The method of any one of claims 1 to 3, wherein the pH of said reducing reagent contacting with the multispecific antigen binding molecule is from about 3 to about 10, preferably pH 6-8.
- [Claim 5] The method of any one of claims 1 to 4, wherein the reducing agent is selected from the group consisting of TCEP, 2-MEA, DTT, Cysteine, GSH and Na₂SO₃, preferably TCEP.
- [Claim 6] The method of any one of claims 1 to 5, wherein the concentration of the reducing agent is from about 0.01 mM to about 100 mM.
- [Claim 7] The method of any one of claims 1 to 6, wherein the concentration of the multispecific antigen binding molecule is from about 0.1 mg/ml to about 50 mg/ml, preferably about 10 mg/ml.
- [Claim 8] The method of any one of claims 1 to 7, further comprising a step of promoting re-oxidization of cysteine disulfide bonds, preferably by removing the reducing agent, preferably by dialysis or buffer exchange.
- [Claim 9] The method of any one of claims 1 to 8, wherein each of the first antigen-binding moiety and the second antigen-binding moiety is capable of binding to CD3 and CD137 but does not bind both CD3 and CD137 at the same time.
- [Claim 10] The method of claim 9, wherein the first antigen-binding moiety and the second antigen-binding moiety each comprises an antibody variable region comprising any one of (a1) to (a17) below:
(a1) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 17, the heavy chain CDR 2 of SEQ ID NO: 31, the heavy chain CDR 3 of SEQ ID NO: 45, the light chain CDR 1 of SEQ ID NO: 64, the light chain CDR 2 of SEQ ID NO: 69 and the light chain CDR 3 of SEQ ID NO: 74;
(a2) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 18, the heavy chain CDR 2 of SEQ ID NO: 32, the heavy chain CDR 3 of SEQ ID NO: 46, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a3) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 19, the heavy chain CDR 2 of SEQ ID NO: 33, the heavy chain CDR 3 of SEQ ID NO: 47, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a4) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 19, the heavy chain CDR 2 of SEQ ID NO: 33, the heavy

chain CDR 3 of SEQ ID NO: 47, the light chain CDR 1 of SEQ ID NO: 65, the light chain CDR 2 of SEQ ID NO: 70 and the light chain CDR 3 of SEQ ID NO: 75;

(a5) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 20, the heavy chain CDR 2 of SEQ ID NO: 34, the heavy chain CDR 3 of SEQ ID NO: 48, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;

(a6) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 22, the heavy chain CDR 2 of SEQ ID NO: 36, the heavy chain CDR 3 of SEQ ID NO: 50, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;

(a7) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 23, the heavy chain CDR 2 of SEQ ID NO: 37, the heavy chain CDR 3 of SEQ ID NO: 51, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;

(a8) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 23, the heavy chain CDR 2 of SEQ ID NO: 37, the heavy chain CDR 3 of SEQ ID NO: 51, the light chain CDR 1 of SEQ ID NO: 66, the light chain CDR 2 of SEQ ID NO: 71 and the light chain CDR 3 of SEQ ID NO: 76;

(a9) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 24, the heavy chain CDR 2 of SEQ ID NO: 38, the heavy chain CDR 3 of SEQ ID NO: 52, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;

(a10) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 25, the heavy chain CDR 2 of SEQ ID NO: 39, the heavy chain CDR 3 of SEQ ID NO: 53, the light chain CDR 1 of SEQ ID NO: 66, the light chain CDR 2 of SEQ ID NO: 71 and the light chain CDR 3 of SEQ ID NO: 76;

(a11) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 26, the heavy chain CDR 2 of SEQ ID NO: 40, the heavy chain CDR 3 of SEQ ID NO: 54, the light chain CDR 1 of SEQ ID NO: 66, the light chain CDR 2 of SEQ ID NO: 71 and the light chain CDR 3 of SEQ ID NO: 76;

(a12) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 26, the heavy chain CDR 2 of SEQ ID NO: 40, the heavy chain CDR 3 of SEQ ID NO: 54, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;

(a13) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 27, the heavy chain CDR 2 of SEQ ID NO: 41, the heavy chain CDR 3 of SEQ ID NO: 55, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;

(a14) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 28, the heavy chain CDR 2 of SEQ ID NO: 42, the heavy chain CDR 3 of SEQ ID NO: 56, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;

(a15) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 82, the heavy chain CDR 2 of SEQ ID NO: 83, the heavy chain CDR 3 of SEQ ID NO: 84, the light chain CDR 1 of SEQ ID NO: 65, the light chain CDR 2 of SEQ ID NO: 70 and the light chain CDR 3 of SEQ ID NO: 75;

(a16) an antibody variable region that binds to the same epitope of any of the antibody variable region selected from (a1) to (a15); and

(a17) an antibody variable fragment that competes with the binding of any of the antibody variable fragment selected from (a1) to (a15).

[Claim 11]

The method of claim 10, wherein the first antigen-binding moiety and the second antigen-binding moiety each comprises an antibody variable region comprising any one of (a1) to (a17) below:

(a1) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 3, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 59;

(a2) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 4, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;

(a3) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 5, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;

(a4) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 5, and a light chain variable region comprising an

amino acid sequence of SEQ ID NO: 60;

(a5) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;

(a6) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 8, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;

(a7) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 9, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;

(a8) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 9, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 61;

(a9) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 10, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;

(a10) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 11, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 61;

(a11) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 12, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 61;

(a12) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 12, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;

(a13) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 13, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;

(a14) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 14, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58; and

(a15) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 81, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 60.

(a16) an antibody variable region that binds to the same epitope of any of the antibody variable region selected from (a1) to (a15); and

(a17) an antibody variable fragment that competes with the binding of any of the antibody variable fragment selected from (a1) to (a15).

- [Claim 12] The method of any one of claims 1 to 11, wherein the third antigen-binding moiety is capable of binding to DLL3, preferably human DLL3.
- [Claim 13] The method of claim 12, wherein the third antigen-binding moiety capable of binding to DLL3 comprises an antibody variable region comprising the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 233, the heavy chain CDR 2 of SEQ ID NO: 234, the heavy chain CDR 3 of SEQ ID NO: 235, the light chain CDR 1 of SEQ ID NO: 237, the light chain CDR 2 of SEQ ID NO: 238 and the light chain CDR 3 of SEQ ID NO: 239
- [Claim 14] The method of claim 13, wherein the third antigen-binding moiety capable of binding to DLL3 comprises an antibody variable region comprising: a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 232, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 236.
- [Claim 15] The method of any one of claims 1 to 14, wherein the multispecific antigen binding molecule further comprises a Fc domain.
- [Claim 16] A preparation of a multispecific antigen binding molecule prepared according to the method of any one of claims 1 to 15, said preparation having a homogeneous population of said multispecific antigen binding molecule having at least one disulfide bond in the CH1 region (position 191 according to EU numbering).
- [Claim 17] A preparation of a multispecific antigen binding molecule prepared according to the method of any one of claims 1 to 15, said preparation having at least 80%, 90%, preferably at least 95% molar ratio of said multispecific antigen binding molecule having at least one disulfide bond in the CH1 region (position 191 according to EU numbering).