plication Pub. No: 2005/0161399, Dillon et al. discusses a reversed-phase LC/MS method of analyzing high molecular weight proteins, including antibodies. In addition, U.S. patent application Pub. No: 2006/194280, Dillon et al. is directed to methods of transiently enriching particular IgG isoforms by subjecting preparations of recombinant IgG proteins with a reduction/oxidation coupling reagent and optionally a chaotropic agent.

Citation List

Patent Literature

[0010]

[PTL 1] WO2012/073985
[PTL 2] WO2015/156268
[PTL 3] WO2014116846

Non Patent Literature

[0011]

[NPL 1] Nat. Biotechnol. (2005) 23, 1073-1078
[NPL 2] Nature. 1985 Apr 18-24;314(6012):628-31.
[NPL 3] Int J Cancer. 1988 Apr 15;41(4):609-15.
[NPL 4] Proc Natl Acad Sci U S A. 1986 Mar;83(5):1453-7.
[NPL 5] Proc Natl Acad Sci U S A. 1995 Jul 18;92(15):7021-5.
[NPL 6] Drug Discov Today. 2005 Sep 15;10(18):1237-44.
[NPL 7] Int J Cancer. 2002 Aug 20;100(6):690-7.
[NPL 8] Cancer Immunol Immunother (2007) 56 (10), 1637-44
[NPL 9] Cancer Immunol Immunother. (2006) 55 (5), 503-14
[NPL 10] Cancer Immunol Immunother. (2009) 58 (1), 95-109
[NPL 11] Nat Rev Drug Discov. 2014 Nov;13(11):799-801.
[NPL 12] Vinay, 2011, Cellular & Molecular Immunology, 8, 281-284
[NPL 13] Houot, 2009, Blood, 114, 3431-8
[NPL 14] Porter, N ENGL J MED, 2011, 365;725-733
[NPL 15] Dubrot, Cancer Immunol. Immunother., 2010, 28, 512-22
[NPL 16] Schabowsky, Vaccine, 2009, 28, 512-22
[NPL 17] Li, Proc Natl Acad Sci USA. 2013, 110(48), 19501-6

Summary ~~of Invention~~

Technical Problem

[0012]

An antibody that exerts both cytotoxic activity mediated by immune cells (e.g. T cells) and activating activity of T cells and/or other immune cells via costimulatory molecules (e.g. CD137) in a target antigen-specific manner while circumventing adverse reactions has not yet been known. An objective of the present ~~invention~~disclosure is to provide antigen-binding molecules which exhibit effective target-specific cell killing efficacy mediated by immune cells (e.g. T cells) while having reduced or minimal side effects.

[0013]

Another objective of the present ~~invention~~disclosure is to provide methods for producing the multispecific antigen-binding molecule, methods for increasing or enriching a

preferred form of multispecific antibody proteins, and methods for eliminating disulfide heterogeneity of such recombinant antibody proteins.

Solution to Problem

[0014]

Antigen-binding molecules which are capable of binding to multiple different antigens (e.g., CD3 on T cells, and CD137 on T cells, NK cells, DC cells, and/or the like), but do not non-specifically crosslink two or more immune cells such as T cells are provided. Such multispecific antigen-binding molecules are capable of modulating and/or activating an immune response while circumventing the cross-linking between different cells (e.g., different T cells) resulting from the binding of a conventional multispecific antigen-binding molecule to antigens expressed on the different cells, which is considered to be responsible for adverse reactions when the multispecific antigen-binding molecule is used as a drug.

[0014a]

In one aspect, the present disclosure provides 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 agent.

[0014b]

In another aspect, the present disclosure refers to a preparation of a multispecific antigen binding molecule prepared according to the method as disclosed herein, 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).

[0014c]

In another aspect, the present disclosure refers to a preparation of a multispecific antigen binding molecule prepared according to the method as disclosed herein, 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).

[0015]

In one ~~aspect~~example, the antigen-binding molecule of the present ~~invention~~disclosure provides new antigen-binding molecules which have very unique structure format(s), which improve or enhance the efficacy of the multispecific antigen-binding molecules. The new antigen-binding molecules with unique structure formats provide the increased number of antigen-binding domains to give the increased valency and/or specificities to respective antigens on effector cells and target cells with the reduced unwanted adverse effects.

[0016]

In a further ~~aspect~~example, one of the antigen-binding molecules having such new unique structure format of the present ~~invention~~disclosure comprises at least two first and second antigen-binding moieties (e.g., Fab domains) which are linked together (e.g., via Fc, disulfide bond, linker, or the like), each of which binds to a first and/or second antigen on effector cells (e.g., immune cells such as T cells, NK cells, DC cells, or the like), and further comprises a third (and optionally a fourth) antigen-binding domain(s) which is linked to any one of the first or second antigen-binding moieties, which bind(s) to the third antigen on target cells (e.g., tumor cells).

[0017]

In a further ~~aspect~~example, one of the antigen-binding molecules having such new unique structure format of the present ~~invention~~disclosure comprises at least a first antigen-binding moiety and a second antigen-binding moiety (e.g., Fab domains) which are linked together (e.g., via Fc, disulfide bond, linker, or the like), each of which binds to a first and/or second antigen on effector cells (e.g., immune cells such as T cells, NK cells, DC cells, or the like), and further comprises a third (and optionally the fourth) antigen-binding moiety(s) which is linked to any one of the first or second antigen-binding moiety, which bind(s) to the third antigen on target cells (e.g., tumor cells), wherein the first and second antigen-binding moieties (e.g. Fab domains) capable of binding to the first antigen and/or a second antigen comprise at least one amino acid mutation(s)

respectively, which create a disulfide linkage between the first and second antigen-binding moieties to hold them close to each other, and, for example, promote cis-antigen binding to the same single effector cell as a result of steric hindrance or shorter distance between the two Dual-Fabs, thereby improving the safety profile of the trispecific antibody (trispecific Ab) by preventing undesirable crosslinking of two CD3/CD137-expressing immune cells mediated by the two Dual-Fabs in an DLL3-independent manner. In one specific ~~aspect~~example, said each of the first antigen-binding moiety and the second antigen-binding moiety is a Fab and comprises at least one cysteine residue (via mutation, substitution or insertion) in the CH1 region, said at least one cysteine residue is capable of forming at least one disulfide bond between the CH1 region of the first antigen-binding moiety and the CH1 region of the second antigen-binding moiety. In another specific ~~aspect~~example, said 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.

[0018]

The antigen-binding molecules having such unique structure formats were surprisingly found to show superior efficacy compared to other multispecific antibody formats (e.g. BiTE) while exhibiting reduced or minimal off-target side-effects attributed by undesired cross-linking among different cells (e.g., effector cells such as T cells). In one ~~aspect~~example, the present ~~invention~~disclosure relates to multispecific antigen-binding molecules that comprise a first antigen-binding moiety and a second antigen-binding moiety, each of which is capable of binding to CD3 and CD137, but does not bind to CD3 and CD137 at the same time (i.e. capable of binding to CD3 and CD137 but not simultaneously); and a third antigen-binding moiety that is capable of binding to DLL3, preferably human DLL3, which induce T-cell dependent cytotoxity more efficiently whilst circumventing adverse toxicity concerns or side effects that other multispecific antigen-binding molecules may have. The present ~~invention~~disclosure provides multispecific antigen-binding molecules and pharmaceutical compositions that can treat various cancers, especially those associated with DLL3 such as DLL3-positive tumors, by comprising the antigen-binding molecule as an active ingredient.

[0019]

In another ~~aspect~~example, the present ~~invention~~disclosure relates to methods of producing the multispecific antigen-binding molecules of novel format comprising one or more disulfide linkage between the first and second antigen-binding moieties (e.g. at the CH1 region); methods for increasing or enriching a preferred form of multispecific antibody protein having said at least one disulfide linkage, and methods for eliminating disulfide heterogeneity of such recombinant antibody proteins by contacting the antibody

preparation with a reducing agent under conditions which allows said at least one disulfide linkage (e.g. at the CH1 region) to form efficiently and properly. In a further ~~aspect~~example, the present ~~invention~~disclosure relates to conformation-specific antibodies that specifically recognize the preferred form of multispecific antibody proteins, and use of the conformation-specific antibodies in purification, analytical or quantification of antibody-containing samples.

[0020]

In one specific ~~aspect~~example, the present disclosure provides the following:

[1] A multispecific antigen-binding molecule comprising:

a first antigen-binding moiety and a second antigen-binding moiety, each of which is capable of binding to CD3 and CD137, but does not bind to CD3 and CD137 at the same time; and

a third antigen-binding moiety that is capable of binding to a third antigen, preferably an antigen expressed on a cancer cell/tissue.

[1A] A multispecific antigen-binding molecule comprising:

a first antigen-binding moiety and a second antigen-binding moiety, each of which is capable of binding to CD3 and CD137, but does not bind to CD3 and CD137 at the same time; and

a third antigen-binding moiety that is capable of binding to DLL3, preferably human DLL3.

[2] The multispecific antigen-binding molecule of any one of [1] to [1A], 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;

[0021]

Another ~~aspect~~example of the present ~~invention~~disclosure relates to:

[1] A method for (i) producing a preparation of a multispecific antigen binding molecule (that has been recombinantly produced by mammalian cells), (ii) purifying a multispecific antigen binding molecule having a desired conformation, or (iii) improving homogeneity of a preparation of a multispecific antigen binding molecule;

wherein the multispecific antigen binding molecule comprises 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 a Fab and 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;

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.

[2] The method of [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.

[3] The method of [1] or [2], wherein said contacting the preparation with a reducing reagent allows and/or facilitates the formation of the 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).

[4] The method of [3], 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).

[5] The method of any one of [1] to [4], wherein the pH of said reducing reagent contacting with the multispecific antigen binding molecule is from about 3 to about 10.

[6] The method of [5], wherein the pH of said reducing reagent contacting with the multispecific antigen binding molecule is about 6, 7 or 8.

(chain 2), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 212 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NO: 215 (chain 4 & chain 5);
(a12) a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 225(chain 1), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 206 (chain 2), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 213 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NO: 215 (chain 4 & chain 5);
(a13) a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 226 (chain 1), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 206 (chain 2), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 213 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NO: 215 (chain 4 & chain 5);
(a14) a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 227 (chain 1), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 206 (chain 2), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 213 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NO: 215 (chain 4 & chain 5); and
(a15) a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 228 (chain 1), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 206 (chain 2), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 231 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NO: 215 (chain 4 & chain 5);
and wherein, preferably the five polypeptide chains (chain 1 to chain 5) connect and/or associate with each other according to the orientation shown in Figure 1(a).
[37] The method of any one of [1] to [36], wherein the fourth polypeptide (chain 4) and the fifth polypeptide (chain 5) are identical.
[38] A preparation of a multispecific antigen binding molecule prepared according to the method of any one of [1] to [37], 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).
[39] A preparation of a multispecific antigen binding molecule prepared according to the method of any one of [1] to [37], said preparation having at least 50%, 60%, 70%, 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).

[0022]

Yet another ~~aspect~~example of the present ~~invention~~disclosure relates to:
[1] A method for producing a multispecific antigen-binding molecule, wherein said

(chain 2), a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 212 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 215 (chain 4 & chain 5);
(a12) a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 225(chain 1), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 206 (chain 2), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 213 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NO: 215 (chain 4 & chain 5);
(a13) a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 226 (chain 1), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 206 (chain 2), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 213 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NO: 215 (chain 4 & chain 5);
(a14) a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 227 (chain 1), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 206 (chain 2), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 213 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NO: 215 (chain 4 & chain 5); and
(a15) a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 228 (chain 1), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 206 (chain 2), a polypeptide chain comprising an amino acid sequence of SEQ ID NO: 231 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NO: 215 (chain 4 & chain 5);
and wherein, preferably the five polypeptide chains (chain 1 to chain 5) connect and/or associate with each other according to the orientation shown in Figure 1(a).
[37] The method of any one of [1] to [36], wherein the fourth polypeptide (chain 4) and the fifth polypeptide (chain 5) are identical.
[38] The method of any one of [1] or [37], wherein only one nucleic acid, or two, three, four or five different nucleic acids encode and express the first, second, third, fourth and fifth polypeptides.

[0023]

In yet another ~~aspect~~example, the present ~~invention~~disclosure relates to:
[1] A method for capturing and/or removing a target antibody from an antibody preparation, comprising the steps of:
a) contacting the antibody preparation comprising the target antibody with an antigen-binding molecule immobilized on a support; and
b) allowing capture of the target antibody by specific binding to the antigen-binding molecule;
wherein said antibody comprises at least two Fabs from an IgG (preferably human

bind to IgG1_001, DualAE05-SG1201 and DualAE05-SG1202k/SG1201hV11 but do not substantially bind to each of IgG4_001 and DualAE05-SG1202 would fulfil the screening criteria to obtain conformation-specific antibodies that specifically bind to CH1 domain of human IgG1 in the "unpaired cysteines" form, but do not bind to CH1 domain of human IgG1 which is in "paired cysteines" form or CH1 domain of human IgG4 CH1. As a result of the screening, four antibodies namely FAB0059ff, FAB0060hh, FAB0133hh, and FAB0135hh were found and selected as conformation-specific anti-CH1 antibodies which specifically bind Dual/LINC 1+2 antibodies in "unpaired cysteines" form (Table 5). As shown in Table 4, these conformation-specific anti-CH1 antibodies exhibit relatively strong binding activities to each of IgG1_001, DualAE05-SG1201 and DualAE05-SG1202k/SG1201hV11 (Relative binding activity >0.8) while exhibiting no or relatively weak binding activities to IgG4_001 (Relative binding activity < 0.1) and DualAE05-SG1202 (Relative binding activity < 0.5).

[0335]

Table 4 shows the relative binding activity of anti-CH1 antibodies to each of tool antibodies DualAE05-SG1201, DualAE05-SG1202, DualAE05-SG1202k/SG1201hV11, IgG1_001 and IgG4_001. The values of relative binding activity to tool antibody (normalized against RU binding value for IgG1_001) are obtained by dividing the Biacore binding response (RU) for anti-CH1 antibody to tool antibody, to RU binding value for anti-CH1 antibody to IgG1_001.

[0336]

[Table 4]

Name of anti-CH1 antibodies	Relative binding activity to tool antibody (normalized against RU binding value for IgG1_001)				
	IgG1_001	IgG4_001	DualAE05-SG1201	DualAE05-SG1202	DualAE05-SG1202k/SG1201hV11
FAB0059ff	1.000	0.007	0.952	0.493	0.929
FAB0060hh	1.000	-0.009	0.968	0.489	0.957
FAB0133hh	1.000	-0.017	1.016	0.206	0.922
FAB0135hh	1.000	-0.023	1.007	0.243	0.960

[0337]

In order to improve the physicochemical properties of the conformation-specific anti-CH1 antibodies, cysteine residues at CDR regions were removed from FAB0059ff, FAB0060hh, and FAB0133hh, and subsequently named as FAB0059Hf/FAB0059L0001, FAB0060Hh/FAB0060L0001, and FAB0133Hh/FAB0133L0001, respectively. Amino acid sequence SEQ ID NOs of the conformation-specific anti-CH1 antibodies were shown in Table 5.

[0338]

[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~~agent.
[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~~ agent 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 Na_2SO_3, 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~~ region that competes with the binding of any of the antibody variable ~~fragment~~ region 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~~ region that competes with the binding of any of the antibody variable ~~fragment~~ region 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).