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## Summary

### Technical Problem

[0015] 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 example is to provide an 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. Another objective of the present example is to provide a pharmaceutical composition comprising the antigen-

binding molecule, and a method for producing the antigen-binding molecule.

### **Solution to Problem**

[0015a] In one aspect, the present disclosure relates to an antigen-binding molecule comprising: (1) a first antigen-binding domain comprising a heavy chain variable (VH) region, a CH1 region, a light chain variable (VL) region, and a light chain constant region (CL); and (2) a second antigen-binding domain comprising a heavy chain variable (VH) region, a CH1 region, a light chain variable (VL) region, and a light chain constant region (CL), and (3) a third antigen-binding domain comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the first antigen-binding domain and the second antigen-binding domain are linked via a first and a second linkage, wherein the first linkage is a Fc region, and wherein the second linkage is

- (i) at least one bond between an amino acid residue in the CH1 region of the first antigen-binding domain and an amino acid residue in the CH1 region of the second antigen-binding domain at position 119, 122, 123, 131, 132, 133, 134, 135, 136, 137, 139, 140, 148, 150, 155, 156, 157, 159, 160, 161, 162, 163, 165, 167, 174, 176, 177, 178, 190, 191, 192, 194, 195, 197, 213, or 214 according to EU numbering, or
- (ii) at least one bond between an amino acid residue in the CL region of the first antigen-binding domain and an amino acid residue in the CL region of the second antigen-binding domain at position 109, 112, 121, 126, 128, 151, 152, 153, 156, 184, 186, 188, 190, 200, 201, 202, 203, 208, 210, 211, 212, or 213 according to EU numbering, wherein the at least one bond is a disulfide bond,

wherein

- (a) the first antigen-binding domain and the second antigen-binding domain are respectively capable of binding to a first antigen and a second antigen which is different from the first antigen, but do not bind to both of the first and second antigens at the same time; or
- (b) the first antigen-binding domain is capable of binding to a first antigen and a second antigen which is different from the first antigen, but does not bind to both of the first and second antigens at the same time, and the second antigen-binding domain is capable of binding to only the second antigen,

wherein the first antigen is a molecule specifically expressed on a T cell and the second antigen is a molecule expressed on a T cell or any other immune cell,

wherein the third antigen-binding domain is capable of binding to a third antigen which is different from the first antigen and the second antigen and is a molecule specifically expressed in a cancer cell the third antigen-binding domain is linked to the first antigen-binding domain through a linkage at any one of the following positions:

- (A) between a C-terminus of a polypeptide comprising the heavy chain variable region (VH) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the heavy chain variable region (VH) of the first antigen-binding domain,
- (B) between a C-terminus of a polypeptide comprising the heavy chain variable region (VH) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the light chain variable region (VL) of the first antigen-binding domain,
- (C) between a C-terminus of a polypeptide comprising the light chain variable region (VL) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the heavy chain variable region (VH) of the first antigen-binding domain, or
- (D) between a C-terminus of a polypeptide comprising the light chain variable region (VL) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the light chain variable region (VL) of the first antigen-binding domain.

[0015b] In another aspect, the present disclosure relates to A method for producing an antigen-binding molecule comprising:

- (a) providing nucleic acids encoding polypeptides that together form:
    - (1) a first antigen-binding domain comprising a heavy chain variable (VH) region and a CH1 region, and a light chain variable (VL) region and a light chain constant region (CL),
    - (2) a second antigen-binding domain comprising a heavy chain variable (VH) region and a CH1 region and a light chain variable (VL) region and a light chain constant region (CL), and
    - (3) a third antigen-binding domain comprising a heavy chain variable (VH) region and a light chain variable (VL) region;
- wherein:
- (i) the first antigen-binding domain and the second antigen-binding domain are respectively capable of binding to a first antigen and a second antigen which is different from the first antigen, but do not bind to both of the first and second antigens at the same time, or
  - (ii) the first antigen-binding domain is capable of binding to a first antigen and a second antigen which is different from the first antigen, but does not bind to both of the first and second antigens at the same time; and the second antigen-binding domain is capable of binding to only the second antigen,
- wherein the first antigen is a molecule specifically expressed on a T cell and the second antigen is a molecule expressed on a T cell or any other immune cell,

wherein the third antigen-binding domain is capable of binding to a third antigen which is different from the first antigen and the second antigen and is a molecule specifically expressed in a cancer cell;

(b) introducing the nucleic acids in (a) into a host cell;

(c) culturing the host cell so that the antigen-binding molecule comprising the polypeptides of (a) is produced,

wherein the first antigen-binding domain and the second antigen-binding domain are linked via a first and a second linkage,

wherein the first linkage is a Fc region, and

wherein the second linkage is at least one bond between an amino acid residue in the CH1 region of the first antigen-binding domain and an amino acid residue in the CH1 region of the second antigen-binding domain at position 119, 122, 123, 131, 132, 133, 134, 135, 136, 137, 139, 140, 148, 150, 155, 156, 157, 159, 160, 161, 162, 163, 165, 167, 174, 176, 177, 178, 190, 191, 192, 194, 195, 197, 213, or 214 according to EU numbering, or between an amino acid residue in the CL region of the first antigen-binding domain and an amino acid residue in the CL region of the second antigen-binding domain at position 109, 112, 121, 126, 128, 151, 152, 153, 156, 184, 186, 188, 190, 200, 201, 202, 203, 208, 210, 211, 212, or 213 according to EU numbering, and wherein the third antigen-binding domain is linked to the first antigen-binding domain through a linkage at any one of the following positions:

(A) between a C-terminus of a polypeptide comprising the heavy chain variable region (VH) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the heavy chain variable region (VH) of the first antigen-binding domain,

(B) between a C-terminus of a polypeptide comprising the heavy chain variable region (VH) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the light chain variable region (VL) of the first antigen-binding domain,

(C) between a C-terminus of a polypeptide comprising the light chain variable region (VL) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the heavy chain variable region (VH) of the first antigen-binding domain, or

(D) between a C-terminus of a polypeptide comprising the light chain variable region (VL) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the light chain variable region (VL) of the first antigen-binding domain; and

(d) obtaining the antigen-binding molecule produced in (c).

[0016] Antigen-binding molecule 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.

[0017] In one example, the antigen-binding molecule of the present example 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.

In a further example, one of the antigen-binding molecule having such new unique structure format of the present example comprises at least two first and second antigen-binding domains (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 domain, which bind(s) to the third antigen on target cells (e.g., tumor cells).

[0018] In a further example, one of the antigen-binding molecule having such new unique structure format of the present example comprises at least two first and second antigen-binding domains (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 domain(s) which is linked to any one of the first or second antigen-binding domain, which bind(s) to the third antigen on target cells (e.g., tumor cells), wherein the first and second antigen-binding domains (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 linkage between the first and second antigen-binding domains to hold them

close to each other, and, for example, promote cis-antigen binding to the same single effector cell.

The antigen-binding molecules having such unique structure formats that the inventors of the present example were surprisingly found to show superior efficacy 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).

[0019] More specifically, the present example relates to the followings.

[1] An antigen-binding molecule comprising at least two antigen-binding domains, which comprises:

(i) a first antigen-binding domain comprising a heavy chain variable (VH) region and a light chain variable (VL) region; and

(ii) a second antigen-binding domain comprising a heavy chain variable (VH) region and a light chain variable (VL) region,

wherein the first antigen-binding domain and the second antigen-binding domain are linked via a Fc region, a disulfide bond or a linker,

wherein the first antigen-binding domain and the second antigen-binding domain are respectively capable of binding to a first antigen and a second antigen which is different from the first antigen, but do not bind to both of the first and second antigens at the same time.

[2] The antigen-binding molecule of [1], which further comprises a third antigen-binding domain comprising a heavy chain variable (VH) region and a light chain variable (VL) region, which is capable of binding to a third antigen which is different from the first antigen and the second antigen,

wherein the third antigen-binding domain is linked to any one of the first antigen-binding domain and the second antigen-binding domain, or a Fc region.

[3] An antigen-binding molecule comprising at least two antigen binding-domains, which comprises:

(i) a first antigen-binding domain comprising a heavy chain variable (VH) region and a light chain variable (VL) region; and

(ii) a second antigen-binding domain comprising a heavy chain variable (VH) region and a light chain variable (VL) region,

wherein the first antigen-binding domain and the second antigen-binding domain are linked via a Fc region, a disulfide bond or a linker,

wherein the first antigen-binding domain is capable of binding to a first antigen and a second antigen which is different from the first antigen, but does not bind to both of the first and second antigens at the same time; and

wherein the second antigen-binding domain is capable of binding to only either one of the first antigen or second antigen.

## CLAIMS

[Claim 1]

An antigen-binding molecule comprising:

- (1) a first antigen-binding domain comprising a heavy chain variable (VH) region, a CH1 region, a light chain variable (VL) region, and a light chain constant region (CL); and
- (2) a second antigen-binding domain comprising a heavy chain variable (VH) region, a CH1 region, a light chain variable (VL) region, and a light chain constant region (CL), and
- (3) a third antigen-binding domain comprising a heavy chain variable (VH) region and a light chain variable (VL) region,

wherein the first antigen-binding domain and the second antigen-binding domain are linked via a first and a second linkage, wherein the first linkage is a Fc region, and wherein the second linkage is

- (i) at least one bond between an amino acid residue in the CH1 region of the first antigen-binding domain and an amino acid residue in the CH1 region of the second antigen-binding domain at position 119, 122, 123, 131, 132, 133, 134, 135, 136, 137, 139, 140, 148, 150, 155, 156, 157, 159, 160, 161, 162, 163, 165, 167, 174, 176, 177, 178, 190, 191, 192, 194, 195, 197, 213, or 214 according to EU numbering, or
- (ii) at least one bond between an amino acid residue in the CL region of the first antigen-binding domain and an amino acid residue in the CL region of the second antigen-binding domain at position 109, 112, 121, 126, 128, 151, 152, 153, 156, 184, 186, 188, 190, 200, 201, 202, 203, 208, 210, 211, 212, or 213 according to EU numbering, wherein the at least one bond is a disulfide bond,

wherein

- (a) the first antigen-binding domain and the second antigen-binding domain are respectively capable of binding to a first antigen and a second antigen which is different from the first antigen, but do not bind to both of the first and second antigens at the same time; or
- (b) the first antigen-binding domain is capable of binding to a first antigen and a second antigen which is different from the first antigen, but does not bind to both of the first and second antigens at the same time, and the second antigen-binding domain is capable of binding to only the second antigen,

wherein the first antigen is a molecule specifically expressed on a T cell and the second antigen is a molecule expressed on a T cell or any other immune cell,

wherein the third antigen-binding domain is capable of binding to a third antigen which is different from the first antigen and the second antigen and is a molecule specifically expressed in a cancer cell the third antigen-binding domain is linked to the first antigen-binding domain through a linkage at any one of the following positions:

- (A) between a C-terminus of a polypeptide comprising the heavy chain variable region (VH) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the heavy chain variable region (VH) of the first antigen-binding domain,
- (B) between a C-terminus of a polypeptide comprising the heavy chain variable region (VH) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the light chain variable region (VL) of the first antigen-binding domain,
- (C) between a C-terminus of a polypeptide comprising the light chain variable region (VL) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the heavy chain variable region (VH) of the first antigen-binding domain, or
- (D) between a C-terminus of a polypeptide comprising the light chain variable region (VL) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the light chain variable region (VL) of the first antigen-binding domain.

[Claim 2]

The antigen-binding molecule of claim 1, wherein the first antigen-binding domain or both the first antigen-binding domain and the second antigen binding domain which is/are capable of binding to a first antigen and a second antigen which is different from the first antigen, but does/do not bind to both of the first and second antigens at the same time, has/have alteration of at least one amino acid, wherein the amino acid to be altered is at least one amino acid selected from Kabat numbering positions 31 to 35, 50 to 65, 71 to 74, and 95 to 102 in an antibody heavy chain variable (VH) region of SEQ ID NO: 184 as a template sequence, and Kabat numbering positions 24 to 34, 50 to 56, and 89 to 97 in an light chain variable (VL) region of SEQ ID NO: 185 as a template sequence.

[Claim 3]

The antigen-binding molecule of claim 1, wherein the first antigen-binding domain or both the first antigen-binding domain and the second antigen binding domain which

is/are capable of binding to a first antigen and a second antigen which is different from the first antigen, but does/do not bind to both of the first and second antigens at the same time, has/have alteration of at least one amino acid, wherein the alteration is substitution of a portion of the amino acid sequence of a VH and/or VL regions binding to the first antigen by an amino acid sequence of a VH and/or VL regions binding to the second antigen, or insertion of an amino acid sequence of a VH and/or VL regions binding to the second antigen into the amino acid sequence of a VH and/or VL regions binding to the first antigen.

[Claim 4]

The antigen-binding molecule of claim 1, wherein the first antigen-binding domain or both the first antigen-binding domain and the second antigen-binding domain which is/are capable of binding to a first antigen and a second antigen which is different from the first antigen, but does/do not bind to both of the first and second antigens at the same time,

wherein the first antigen-binding domain or both the first antigen-binding domain and the second antigen-binding domain comprises/comprise a VH region and a VL region selected from the group consisting of (I) to (IX):

(I) a VH region comprising a heavy chain complementarity determining region (CDR) 1 comprising SEQ ID NO: 12, a heavy chain CDR 2 comprising SEQ ID NO: 23, a heavy chain CDR 3 comprising SEQ ID NO: 34, and a VL region comprising a light chain CDR 1 comprising SEQ ID NO: 49, a light chain CDR 2 comprising SEQ ID NO: 53, and a light chain CDR 3 comprising SEQ ID NO: 57;

(II) a VH region comprising a heavy chain CDR 1 comprising SEQ ID NO: 13, a heavy chain CDR 2 comprising SEQ ID NO: 24, a heavy chain CDR 3 comprising SEQ ID NO: 35, and a VL region comprising a light chain CDR 1 comprising SEQ ID NO: 50, a light chain CDR 2 comprising SEQ ID NO: 54, and a light chain CDR 3 comprising SEQ ID NO: 58;

(III) a VH region comprising a heavy chain CDR 1 comprising SEQ ID NO: 14, a heavy chain CDR 2 comprising SEQ ID NO: 25, a heavy chain CDR 3 comprising SEQ ID NO: 36, and a VL region comprising a light chain CDR 1 comprising SEQ ID NO: 49, a light chain CDR 2 comprising SEQ ID NO: 53, and a light chain CDR 3 comprising SEQ ID NO: 57;

(IV) a VH region comprising a heavy chain CDR 1 comprising SEQ ID NO: 15, a heavy chain CDR 2 comprising SEQ ID NO: 26, a heavy chain CDR 3 comprising SEQ ID NO: 37, and a VL region comprising a light chain CDR 1 comprising SEQ ID NO:

49, a light chain CDR 2 comprising SEQ ID NO: 53, and a light chain CDR 3 comprising SEQ ID NO: 57;

(V) a VH region comprising a heavy chain CDR 1 comprising SEQ ID NO: 16, a heavy chain CDR 2 comprising SEQ ID NO: 27, a heavy chain CDR 3 comprising SEQ ID NO: 38, and a VL region comprising a light chain CDR 1 comprising SEQ ID NO: 49, a light chain CDR 2 comprising SEQ ID NO: 53, and a light chain CDR 3 comprising SEQ ID NO: 57;

(VI) a VH region comprising a heavy chain CDR 1 comprising SEQ ID NO: 17, a heavy chain CDR 2 comprising SEQ ID NO: 28, a heavy chain CDR 3 comprising SEQ ID NO: 39, and a VL region comprising a light chain CDR 1 comprising SEQ ID NO: 49, a light chain CDR 2 comprising SEQ ID NO: 53, and a light chain CDR 3 comprising SEQ ID NO: 57;

(VII) a VH region comprising a heavy chain CDR 1 comprising SEQ ID NO: 18, a heavy chain CDR 2 comprising SEQ ID NO: 29, a heavy chain CDR 3 comprising SEQ ID NO: 40, and a VL region comprising a light chain CDR 1 comprising SEQ ID NO: 49, a light chain CDR 2 comprising SEQ ID NO: 53, and a light chain CDR 3 comprising SEQ ID NO: 57;

(VIII) a VH region comprising a heavy chain CDR 1 comprising SEQ ID NO: 19, a heavy chain CDR 2 comprising SEQ ID NO: 30, a heavy chain CDR 3 comprising SEQ ID NO: 41, and a VL region comprising a light chain CDR 1 comprising SEQ ID NO: 49, a light chain CDR 2 comprising SEQ ID NO: 53, and a light chain CDR 3 comprising SEQ ID NO: 57; and

(IX) a VH region comprising a heavy chain CDR 1 comprising SEQ ID NO: 20, a heavy chain CDR 2 comprising SEQ ID NO: 31, a heavy chain CDR 3 comprising SEQ ID NO: 42, and a VL region comprising a light chain CDR 1 comprising SEQ ID NO: 49, a light chain CDR 2 comprising SEQ ID NO: 53, and a light chain CDR 3 comprising SEQ ID NO: 57.

[Claim 5]

The antigen-binding molecule of any one of claims 1 to 4, wherein the Fc region is a Fc region having reduced binding activity against Fc gamma R as compared with that of the Fc region of a wild-type human IgG1 antibody.

[Claim 6]

The antigen-binding molecule of any one of claims 1 to 5, wherein the first antigen-binding domain comprises a heavy chain hinge region and the second antigen-binding

domain comprises a heavy chain hinge region respectively, and the first antigen-binding domain and the second antigen-binding domain are linked to each other by one or more native disulfide bonds in the respective hinge regions.

[Claim 7]

The antigen-binding molecule of any one of claims 1 to 6, wherein the amino acid residue at position 191 according to EU numbering in the respective CH1 region of the first antigen-binding domain and the second antigen-binding domain are linked with each other.

[Claim 8]

The antigen-binding molecule of any one of claims 1 to 7, wherein the first antigen is CD3 and the second antigen is CD137.

[Claim 9]

A method for producing an antigen-binding molecule comprising:

(a) providing nucleic acids encoding polypeptides that together form:

- (1) a first antigen-binding domain comprising a heavy chain variable (VH) region and a CH1 region, and a light chain variable (VL) region and a light chain constant region (CL),
- (2) a second antigen-binding domain comprising a heavy chain variable (VH) region and a CH1 region and a light chain variable (VL) region and a light chain constant region (CL), and
- (3) a third antigen-binding domain comprising a heavy chain variable (VH) region and a light chain variable (VL) region;

wherein:

- (i) the first antigen-binding domain and the second antigen-binding domain are respectively capable of binding to a first antigen and a second antigen which is different from the first antigen, but do not bind to both of the first and second antigens at the same time, or
- (ii) the first antigen-binding domain is capable of binding to a first antigen and a second antigen which is different from the first antigen, but does not bind to both of the first and second antigens at the same time; and the second antigen-binding domain is capable of binding to only the second antigen,

wherein the first antigen is a molecule specifically expressed on a T cell and the second antigen is a molecule expressed on a T cell or any other immune cell,

wherein the third antigen-binding domain is capable of binding to a third antigen which is different from the first antigen and the second antigen and is a molecule specifically expressed in a cancer cell;

(b) introducing the nucleic acids in (a) into a host cell;

(c) culturing the host cell so that the antigen-binding molecule comprising the polypeptides of (a) is produced,

wherein the first antigen-binding domain and the second antigen-binding domain are linked via a first and a second linkage,

wherein the first linkage is a Fc region, and

wherein the second linkage is at least one bond between an amino acid residue in the CH1 region of the first antigen-binding domain and an amino acid residue in the CH1 region of the second antigen-binding domain at position 119, 122, 123, 131, 132, 133, 134, 135, 136, 137, 139, 140, 148, 150, 155, 156, 157, 159, 160, 161, 162, 163, 165, 167, 174, 176, 177, 178, 190, 191, 192, 194, 195, 197, 213, or 214 according to EU numbering, or between an amino acid residue in the CL region of the first antigen-binding domain and an amino acid residue in the CL region of the second antigen-binding domain at position 109, 112, 121, 126, 128, 151, 152, 153, 156, 184, 186, 188, 190, 200, 201, 202, 203, 208, 210, 211, 212, or 213 according to EU numbering, and wherein the third antigen-binding domain is linked to the first antigen-binding domain through a linkage at any one of the following positions:

- (A) between a C-terminus of a polypeptide comprising the heavy chain variable region (VH) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the heavy chain variable region (VH) of the first antigen-binding domain,
- (B) between a C-terminus of a polypeptide comprising the heavy chain variable region (VH) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the light chain variable region (VL) of the first antigen-binding domain,
- (C) between a C-terminus of a polypeptide comprising the light chain variable region (VL) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the heavy chain variable region (VH) of the first antigen-binding domain, or

- (D) between a C-terminus of a polypeptide comprising the light chain variable region (VL) of the third antigen-binding domain and an N-terminus of a polypeptide comprising the light chain variable region (VL) of the first antigen-binding domain; and
- (d) obtaining the antigen-binding molecule produced in (c).

[Claim 10]

The method of claim 9, wherein the provision of the antigen-binding domain that does not bind to the first antigen and the second antigen at the same time as defined in (i) and (ii) comprises:

- preparing a library of the antigen-binding domain with at least one amino acid altered in their heavy chain variable (VH) region and light chain variable (VL) region, each of which binds to the first antigen or the second antigen, wherein the altered variable regions differ in at least one amino acid from each other and wherein the alteration is alteration of at least one amino acid selected from Kabat numbering positions 31 to 35, 50 to 65, 71 to 74, and 95 to 102 in the heavy chain variable (VH) region of SEQ ID NO: 184 as a template sequence, and Kabat numbering positions 24 to 34, 50 to 56, and 89 to 97 in the light chain variable (VL) region of SEQ ID NO: 185 as a template sequence; and
- selecting, from the prepared library, an antigen-binding domain comprising a heavy chain variable (VH) region and a light chain variable (VL) region that has binding activity against the first antigen and the second antigen, but does not bind to the first antigen and the second antigen at the same time.

[Claim 11]

The method of claim 9, wherein the provision of the antigen-binding domain that does not bind to the first antigen and the second antigen at the same time as defined in (i) and (ii) comprises:

- preparing a library of the antigen-binding domain with at least one amino acid altered in their heavy chain variable (VH) region and light chain variable (VL) region, each of which binds to the first antigen or the second antigen, wherein the altered variable regions differ in at least one amino acid from each other and wherein the alteration is substitution of a portion of the amino acid sequence of a VH and/or VL regions binding to the first antigen by an amino acid sequence of a VH and/or VL regions binding to the second antigen, or insertion of an amino acid sequence of a VH and/or VL regions

binding to the second antigen into the amino acid sequence of a VH and/or VL regions binding to the first antigen; and

- selecting, from the prepared library, an antigen-binding domain comprising a heavy chain variable (VH) region and a light chain variable (VL) region that has binding activity against the first antigen and the second antigen, but does not bind to the first antigen and the second antigen at the same time.

[Claim 12]

The method of any one of claims 9 to 11, wherein the first antigen-binding domain comprises a heavy chain hinge region and the second antigen-binding domain comprises a heavy chain hinge region respectively, and in said first linkage the first antigen-binding domain and the second antigen-binding domain are linked to each other by one or more native disulfide bonds in the respective hinge regions.