

Claims

- [Claim 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 human CD3 and human CD137,
but does not bind to human CD3 and human CD137 at the same time;
and
a third antigen-binding moiety which binds to human DLL3.
- [Claim 2] The multispecific antigen-binding molecule of claim 1, wherein the
first antigen-binding moiety and the second antigen-binding moiety
each comprises an antibody variable region comprising any one of (a1)
to (a15) 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; and

(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.

[Claim 3]

The multispecific antigen-binding molecule of any one of claims 1 to 2, wherein the first antigen-binding moiety and the second antigen-binding moiety each comprises an antibody variable region comprising any one of (a1) to (a15) 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.

[Claim 4] The multispecific antigen-binding molecule of any one of claims 1 to 3, wherein each of the first antigen-binding moiety and the second antigen-binding moiety is a Fab molecule and comprises at least one disulfide bond formed between the CH1 region of the first antigen-binding moiety and the CH1 region of the second antigen-binding moiety.

[Claim 5] The multispecific antigen-binding molecule of claim 4, wherein each of the first antigen-binding moiety and the second antigen-binding moiety is a Fab molecule and comprises one disulfide bond formed between the amino acid residues at position 191 according to EU numbering in the respective CH1 region of the first antigen-binding moiety and the second antigen-binding moiety.

[Claim 6] The multispecific antigen-binding molecule of any one of claims 1 to 5, wherein each of the first, second and third antigen binding moiety is a Fab molecule, wherein the third antigen binding moiety is fused at the C-terminus of the Fab heavy chain (CH1) to the N-terminus of the Fab

- heavy chain of either one of the first antigen binding moiety or the second antigen binding moiety, optionally via a peptide linker.
- [Claim 7] The multispecific antigen-binding molecule of claim 6, wherein said peptide linker is selected from the group consisting of the amino acid sequence of SEQ ID NO: 248, SEQ ID NO: 249 or SEQ ID NO: 259.
- [Claim 8] The multispecific antigen-binding molecule of any one of claims 1 to 7, wherein the third antigen binding moiety is a crossover Fab molecule in which the variable regions of the Fab light chain and the Fab heavy chain are exchanged, and wherein each of the first and second antigen binding moiety is a conventional Fab molecule.
- [Claim 9] The multispecific antigen-binding molecule of claim 8, wherein in the constant domain CL of the light chain of each of the first and second antigen binding moiety, the amino acids at position 123 and 124 are arginine (R) and lysine (K) respectively (numbering according to Kabat), and wherein in the constant domain CH1 of the heavy chain of each of the first and second antigen binding moiety the amino acids at position 147 and 213 are glutamic acid (E) (numbering according to EU numbering).
- [Claim 10] The multispecific antigen-binding molecule of any one of claims 1 to 9, wherein the third antigen-binding moiety comprises an antibody variable region comprising a heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 233, a heavy chain CDR 2 of SEQ ID NO: 234, a heavy chain CDR 3 of SEQ ID NO: 235, a light chain CDR 1 of SEQ ID NO: 237, a light chain CDR 2 of SEQ ID NO: 238 and a light chain CDR 3 of SEQ ID NO: 239.
- [Claim 11] The multispecific antigen-binding molecule of any one of claims 1 to 10, wherein the third antigen-binding moiety 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 12] The multispecific antigen-binding molecule of any one of claims 1 to 11, further comprises a Fc domain.
- [Claim 13] A multispecific antigen-binding molecule comprising five polypeptide chains in any one of the combination selected from (a1) to (a15) below: (a1) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 201 (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 NOs: 208 (chain 3) and

two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 214 (chain 4 & chain 5);

(a2) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 203 (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 NOs: 209 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 214 (chain 4 & chain 5);

(a3) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 204 (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 NOs: 209 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 214 (chain 4 & chain 5);

(a4) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 205 (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 NOs: 209 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 214 (chain 4 & chain 5);

(a5) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 216 (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 NOs: 229 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 214 (chain 4 & chain 5);

(a6) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 217 (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 NOs: 210 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 214 (chain 4 & chain 5);

(a7) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 219 (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 NOs: 211 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 214 (chain 4 & chain 5);

(a8) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 220 (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 NOs: 211 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 214 (chain 4 & chain 5);

(a9) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 221 (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 NOs: 211 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 214 (chain 4 & chain 5);

(a10) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 222 (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 NOs: 230 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 214 (chain 4 & chain 5);

(a11) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 223 (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 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 NOs: 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 NOs: 213 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 215 (chain 4 & chain 5);

(a13) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 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 NOs: 213 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 215 (chain 4 & chain 5);

(a14) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 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 NOs: 213 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 215 (chain 4 & chain 5); and
(a15) a polypeptide chain comprising an amino acid sequence of SEQ ID NOs: 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 NOs: 231 (chain 3) and two polypeptide chains each comprising an amino acid sequence of SEQ ID NOs: 215 (chain 4 & chain 5).

- [Claim 14] An isolated polynucleotide or plurality of polynucleotides encoding the multispecific antigen-binding molecule of any one of claims 1 to 13.
- [Claim 15] A host cell comprising the polynucleotide or plurality of polynucleotides of claim 14.
- [Claim 16] A method of producing the multispecific antigen-binding molecule of any one of claims 1 to 13, comprising the steps of a) culturing the host cell of claim 15 under conditions suitable for the expression of the antigen-binding molecule and b) recovering the antigen-binding molecule.