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[Summary ~~of Invention~~]

[0013a]

In one aspect, the present disclosure provides an antigen-binding molecule comprising an antibody variable region that is capable of binding to CD3 and CD137, and bind to either of CD3 or CD137, wherein the antigen-binding molecule has an agonistic activity against CD137 and wherein the antibody variable region comprises any one of the following:

- (a1) a heavy chain complementarity determining region 1 (HCDR1) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 16, a heavy chain complementarity determining region 2 (HCDR2) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 30, a heavy chain complementarity determining region 3 (HCDR3) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 44, a light chain complementarity determining region 1 (LCDR1) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 63, a light chain complementarity determining region 2 (LCDR2) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 68, and a light chain complementarity determining region 3 (LCDR3) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 73;
- (a2) a heavy chain complementarity determining region 1 (HCDR1) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 17, a heavy chain complementarity determining region 2 (HCDR2) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 31, a heavy chain complementarity determining region 3 (HCDR3) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 45, a light chain complementarity determining region 1 (LCDR1) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 64, a light chain complementarity determining region 2 (LCDR2) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 69, and a light chain complementarity determining region 3 (LCDR3) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 74;

- [illegible]

that is at least 70%, 80% or 90% identical to SEQ ID NO: 63, a light chain complementarity determining region 2 (LCDR2) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 68, and a light chain complementarity determining region 3 (LCDR3) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 73;

- [illegible]

- sequence of SEQ ID NO: 63, a LCDR2 comprising an amino acid sequence of SEQ ID NO: 68, and a LCDR3 comprising an amino acid sequence of SEQ ID NO: 73;
- (b14) a HCDR1 comprising an amino acid sequence of SEQ ID NO: 27, a HCDR2 comprising an amino acid sequence of SEQ ID NO: 41, a HCDR3 comprising an amino acid sequence of SEQ ID NO: 55, a LCDR1 comprising an amino acid sequence of SEQ ID NO: 63, a LCDR2 comprising an amino acid sequence of SEQ ID NO: 68, and a LCDR3 comprising an amino acid sequence of SEQ ID NO: 73;
- (b15) a HCDR1 comprising an amino acid sequence of SEQ ID NO: 28, a HCDR2 comprising an amino acid sequence of SEQ ID NO: 42, a HCDR3 comprising an amino acid sequence of SEQ ID NO: 56, a LCDR1 comprising an amino acid sequence of SEQ ID NO: 63, a LCDR2 comprising an amino acid sequence of SEQ ID NO: 68, and a LCDR3 comprising an amino acid sequence of SEQ ID NO: 73;
- (e) an antibody variable region that competes for binding to CD3 with any one of the antibody variable regions of (a1) to (b15);
- (f) an antibody variable region that competes for binding to CD137 with any one of the antibody variable regions of (a1) to (b15);
- (g) an antibody variable region that binds to the same epitope on CD3 with any one of the antibody variable regions of (a1) to (b15);
- (h) an antibody variable region that binds to the same epitope on CD137 with any one of the antibody variable regions of (a1) to (b15).

[0013b]

In another aspect, the present disclosure provides an antigen-binding molecule comprising an antibody variable region that is capable of binding to CD3 and CD137, and bind to either of CD3 or CD137, wherein the antigen-binding molecule has an agonistic activity against CD137 and wherein the antibody variable region comprises any one of the following:

- (c1) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 2, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c2) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 3, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 59;
- (c3) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 4, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c4) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 5, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c5) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 5, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 60;
- (c6) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 6, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c7) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 8, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c8) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 9, and a light chain variable domain

- (d13) a heavy chain variable domain (VH) of SEQ ID NO: 12, and a light chain variable domain (VL) of SEQ ID NO: 58;
- (d14) a heavy chain variable domain (VH) of SEQ ID NO: 13, and a light chain variable domain (VL) of SEQ ID NO: 58;
- (d15) a heavy chain variable domain (VH) of SEQ ID NO: 14, and a light chain variable domain (VL) of SEQ ID NO: 58;
- (e) an antibody variable region that competes for binding to CD3 with any one of the antibody variable regions of (c1) to (d15);
- (f) an antibody variable region that competes for binding to CD137 with any one of the antibody variable regions of (c1) to (d15);
- (g) an antibody variable region that binds to the same epitope on CD3 with any one of the antibody variable regions of (c1) to (d15);
- (h) an antibody variable region that binds to the same epitope on CD137 with any one of the antibody variable regions of (c1) to (d15).

[0013c]

In another aspect, the present disclosure provides an isolated polynucleotide comprising a nucleotide sequence that encodes the antigen-binding molecule as described herein.

[0013d]

In another aspect, the present disclosure provides an expression vector comprising the polynucleotide as described herein.

[0013e]

In another aspect, the present disclosure provides a host cell transformed or transfected with the polynucleotide as described herein or the expression vector as described herein.

[0013f]

In another aspect, the present disclosure provides a method of producing a multispecific antigen-binding molecule or a multispecific antibody, comprising culturing the host cell as described herein.

[0013g]

In another aspect, the present disclosure provides a multispecific antigen-binding molecule or a multispecific antibody as obtainable by the method as described herein.

[0013h]

In another aspect, the present disclosure provides a method of obtaining or screening for an antibody variable region that is capable of binding to CD3 and CD137, and bind to either of CD3 or CD137, comprising:

- (a) providing a library comprising a plurality of antibody variable region,
- (b) contacting the library provided in step (a) with either CD3 or CD137 as a first antigen and collecting antibody variable regions bound to the first antigen,
- (c) contacting the antibody variable regions collected in step (b) with a second antigen out of CD3 and CD137 and collecting antibody variable regions bound to the second antigen, and
- (d) selecting an antibody variable region which:
 - (1) has an agonistic activity against CD137 and binds to CD137 with an equilibrium dissociation constant (KD) of less than about 5×10^{-6} M or between 5×10^{-6} M and 3×10^{-8} M, preferably as measured by SPR at the following condition: 37 degrees C, pH 7.4, 20 mM ACES, 150 mM NaCl, 0.05% Tween 20, 0.005% NaN₃; the antigen-binding molecule is immobilized on a CM4 sensor chip, the antigen serves as analyte; and/or
 - (2) binds to CD3 with an equilibrium dissociation constant (KD) of between 2×10^{-6} M and 1×10^{-8} M, preferably as measured by SPR at the following condition: 25 degrees C, pH 7.4, 20 mM ACES, 150 mM NaCl, 0.05% Tween 20, 0.005% NaN₃; the antigen-binding molecule is immobilized on a CM4 sensor chip, the antigen serves as analyte.

[0013i]

In another aspect, the present disclosure provides a pharmaceutical composition comprising the antigen-binding molecule as described herein, or the multispecific antigen-binding molecule or multispecific antibody as described herein, and a pharmaceutically acceptable carrier.

[0013j]

In another aspect, the present disclosure provides the pharmaceutical composition as described herein or the antigen-binding molecule as described herein for use in the treatment of cancer. In another aspect, the present disclosure provides a use of the pharmaceutical composition as described herein or the antigen-binding molecule as described herein in the manufacture of a medicament for treating cancer. In another aspect, the present disclosure provides a use of the pharmaceutical composition as described herein or the antigen-binding molecule as described herein in the manufacture of a medicament for preventing, treating or inhibiting cancer in a mammalian subject suffering from cancer. In another aspect, the present disclosure provides a use of the pharmaceutical composition as described herein or the antigen-binding molecule as described herein in the manufacture of a medicament for inducing cytotoxicity, preferably T-cell dependent cytotoxicity in a mammalian subject suffering from cancer. In another aspect, the present disclosure provides a use of the pharmaceutical composition as described herein or the antigen-binding molecule as described herein for reducing or killing cancer cell in a mammalian subject suffering from cancer. In another aspect, the present disclosure provides a use of the pharmaceutical composition as described herein or the antigen-binding as described herein for extending lifespan or survival rate of a cancer in a mammalian subject suffering from cancer.

[Technical Problem]

[0014]

Tri-specific antibodies comprising a tumor-specific antigen (EGFR)-binding domain, a CD137-binding domain, and a CD3-binding domain were already reported (WO2014116846). However, since antibodies with such a molecular format can bind to three different antigens at the same time, the present inventors speculated that those tri-specific antibodies could result in cross-linking between CD3 epsilon -expressing T cells and CD137-expressing cells (e.g. T cells, B cells, NK cells, DCs etc.) by binding to CD3 and CD137 at the same time. Furthermore, it was already reported that bispecific antibodies against CD8 and CD3 epsilon induced mutual cytotoxicity among CD8 positive T cells because the antibodies cross-linked them (Wong, Clin. Immunol. Immunopathol. 1991, 58(2), 236-250). Therefore, the present inventors speculated that bispecific antibodies against a molecule expressed on T cells and CD3 epsilon would also induce mutual cytotoxicity among T cells because they would cross-link cells expressing the molecule and

Claims

1. ~~An antigen-binding molecule comprising:
an antibody variable region that is capable of binding to CD3 and CD137, but does not bind to CD3 and CD137 at the same time; wherein the antigen-binding molecule binds to CD137 with an equilibrium dissociation constant (KD) of less than 5×10^{-6} M, preferably as measured by SPR at the following condition:
37 degrees C, pH 7.4, 20 mM ACES, 150 mM NaCl, 0.05% Tween 20, 0.005% NaN₃;
the antigen-binding molecule is immobilized on a CM4 sensor chip, the antigen serves as analyte.~~
2. ~~The antigen-binding molecule of claim 1, wherein the antigen-binding molecule binds to:
(a) ~~at least one, two, three or more amino acid residues of the extracellular domain of CD3 epsilon (CD3 epsilon) comprising the amino acid sequence of SEQ ID NO: 159; and/or~~
(b) ~~at least one, two, three or more amino acid residues of the N-terminal region of CD137 comprising the amino acid sequence of LQDPCSNCPAGTFCDNNRNQICSPCPPNSFSSAGGQRTCDICRQCKGVF RTRKECSSTSNAEC (SEQ ID NO: 152), preferably LQDPCSN, NNNRNQI and/or GQRTCDI of human CD137.~~~~
13. The An antigen-binding molecule comprising an antibody variable region that is capable of binding to CD3 and CD137, and bind to either of CD3 or CD137, wherein the antigen-binding molecule has an agonistic activity against CD137 and ~~of claim 1 or 2,~~ wherein the antibody variable region comprises any one of the following:
 - (a1) a heavy chain complementarity determining region 1 (HCDR1) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 16, a heavy chain complementarity determining region 2 (HCDR2) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 30, a heavy chain complementarity determining region 3 (HCDR3) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 44, a light chain complementarity determining region 1 (LCDR1) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 63, a light chain complementarity determining region 2 (LCDR2) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 68, and a light chain complementarity determining region 3 (LCDR3) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 73;
 - (a2) a heavy chain complementarity determining region 1 (HCDR1) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 17, a heavy chain complementarity determining region 2 (HCDR2) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 31, a heavy chain complementarity determining region 3 (HCDR3) comprising an amino acid sequence that is at least 70%, 80% or 90% identical

[illegible]

- [illegible]

an amino acid sequence of SEQ ID NO: 51, a LCDRI comprising an amino acid sequence of SEQ ID NO: 63, a LCDR2 comprising an amino acid sequence of SEQ ID NO: 68, and a LCDR3 comprising an amino acid sequence of SEQ ID NO: 73;

- [illegible]

sequence of SEQ ID NO: 68, and a LCDR3 comprising an amino acid sequence of SEQ ID NO: 73;

- (e) an antibody variable region that competes for binding to CD3 with any one of the antibody variable regions of (a1) to (b15);
- (f) an antibody variable region that competes for binding to CD137 with any one of the antibody variable regions of (a1) to (b15);
- (g) an antibody variable region that binds to the same epitope on CD3 with any one of the antibody variable regions of (a1) to (b15);
- (h) an antibody variable region that binds to the same epitope on CD137 with any one of the antibody variable regions of (a1) to (b15).

2. An antigen-binding molecule comprising an antibody variable region that is capable of binding to CD3 and CD137, and bind to either of CD3 or CD137, wherein the antigen-binding molecule has an agonistic activity against CD137 and wherein the antibody variable region comprises any one of the following:

- (c1) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 2, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c2) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 3, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 59;
- (c3) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 4, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c4) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 5, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c5) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 5, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 60;
- (c6) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 6, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c7) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 8, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;

- (c8) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 9, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c9) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 9, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 61;
- (c10) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 10, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c11) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 11, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 61;
- (c12) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 12, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 61;
- (c13) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 12, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c14) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 13, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (c15) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 14, and a light chain variable domain (VL) comprising an amino acid sequence that is at least 70%, 80% or 90% identical to SEQ ID NO: 58;
- (d1) a heavy chain variable domain (VH) of SEQ ID NO: 2, and a light chain variable domain (VL) of SEQ ID NO: 58;
- (d2) a heavy chain variable domain (VH) of SEQ ID NO: 3, and a light chain variable domain (VL) of SEQ ID NO: 59;
- (d3) a heavy chain variable domain (VH) of SEQ ID NO: 4, and a light chain variable domain (VL) of SEQ ID NO: 58;
- (d4) a heavy chain variable domain (VH) of SEQ ID NO: 5, and a light chain variable domain (VL) of SEQ ID NO: 58;
- (d5) a heavy chain variable domain (VH) of SEQ ID NO: 5, and a light chain variable domain (VL) of SEQ ID NO: 60;
- (d6) a heavy chain variable domain (VH) of SEQ ID NO: 6, and a light chain variable domain (VL) of SEQ ID NO: 58;

- (d7) a heavy chain variable domain (VH) of SEQ ID NO: 8, and a light chain variable domain (VL) of SEQ ID NO: 58;
- (d8) a heavy chain variable domain (VH) of SEQ ID NO: 9, and a light chain variable domain (VL) of SEQ ID NO: 58;
- (d9) a heavy chain variable domain (VH) of SEQ ID NO: 9, and a light chain variable domain (VL) of SEQ ID NO: 61;
- (d10) a heavy chain variable domain (VH) of SEQ ID NO: 10, and a light chain variable domain (VL) of SEQ ID NO: 58;
- (d11) a heavy chain variable domain (VH) of SEQ ID NO: 11, and a light chain variable domain (VL) of SEQ ID NO: 61;
- (d12) a heavy chain variable domain (VH) of SEQ ID NO: 12, and a light chain variable domain (VL) of SEQ ID NO: 61;
- (d13) a heavy chain variable domain (VH) of SEQ ID NO: 12, and a light chain variable domain (VL) of SEQ ID NO: 58;
- (d14) a heavy chain variable domain (VH) of SEQ ID NO: 13, and a light chain variable domain (VL) of SEQ ID NO: 58;
- (d15) a heavy chain variable domain (VH) of SEQ ID NO: 14, and a light chain variable domain (VL) of SEQ ID NO: 58;
- (e) an antibody variable region that competes for binding to CD3 with any one of the antibody variable regions of (ca1) to (d15);
- (f) an antibody variable region that competes for binding to CD137 with any one of the antibody variable regions of (ca1) to (d15);
- (g) an antibody variable region that binds to the same epitope on CD3 with any one of the antibody variable regions of (ca1) to (d15);
- (h) an antibody variable region that binds to the same epitope on CD137 with any one of the antibody variable regions of (ca1) to (d15).

3. The antigen-binding molecule of claim 1 or 2, wherein the antigen-binding molecule binds to CD137 with an equilibrium dissociation constant (KD) of less than 5×10^{-6} M, preferably as measured by SPR at the following condition:

37 degrees C, pH 7.4, 20 mM ACES, 150 mM NaCl, 0.05% Tween 20, 0.005% NaN₃; the antigen-binding molecule is immobilized on a CM4 sensor chip, the antigen serves as analyte.

4. The antigen-binding molecule of any of claims 1 to 3, wherein the antigen-binding molecule binds to:

- (a) at least one, two, three or more amino acid residues of the extracellular domain of CD3 epsilon (CD3 epsilon) comprising the amino acid sequence of SEQ ID NO: 159; and/or
- (b) at least one, two, three or more amino acid residues of the N-terminal region of CD137 comprising the amino acid sequence of LQDPCSNCPAGTFCDNNRNQICSPCPPNSFSSAGGQRTCDICRQCKGVF RTRKECSSTSNAEC (SEQ ID NO: 152), preferably LQDPCSN, NNRNQI and/or GQRTCDI of human CD137.

54. The antigen-binding molecule of any one of claim ~~13~~ (a1)-(a15) or claim 2 (c1)-(c15), which comprises:

- (a) a heavy chain variable domain amino acid sequence comprising, at each of the following positions (all by Kabat numbering), one or more of the following amino acid residues indicated for that position:
- A, D, E, I, G, K, L, M, N, R, T, W or Y at the amino acid position 26;
 - D, F, G, I, M or L, at the amino acid position 27;
 - D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 28;
 - F or W at the amino acid position 29;
 - A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 30;
 - F, I, N, R, S, T or V at the amino acid position 31;
 - A, H, I, K, L, N, Q, R, S, T or V at the amino acid position 32;
 - W at the amino acid position 33;
 - F, I, L, M or V at the amino acid position 34;
 - F, H, S, T, V or Y at the amino acid position 35;
 - E, F, H, I, K, L, M, N, Q, S, T, W or Y at the amino acid position 50;
 - I, K or V at the amino acid position 51;
 - K, M, R, or T at the amino acid position 52;
 - A, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W or Y at the amino acid position 52b;
 - A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 52c;
 - A, E, F, H, K, L, M, N, Q, R, S, T, V, W or Y at the amino acid position 53;
 - A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W or Y at the amino acid position 54;
 - E, F, G, H, L, M, N, Q, W or Y at the amino acid position 55;
 - A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W or Y at the amino acid position 56;
 - A, D, E, G, H, I, K, L, M, N, P, Q, R, S, T or V at the amino acid position 57;
 - A, F, H, K, N, P, R or Y at the amino acid position 58;
 - A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 59;
 - A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 60;
 - A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 61;
 - A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 62;
 - A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 63;

A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 64;

A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 65;

H or R at the amino acid position 93;

F, G, H, L, M, S, T, V or Y at the amino acid position 94;

I or V at the amino acid position 95;

F, H, I, K, L, M, T, V, W or Y at the amino acid position 96;

F, Y or W at the amino acid position 97;

A, F, G, H, I, K, L, M, N, Q, R, S, T, V, W or Y at the amino acid position 98;

A, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 99;

A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 100;

A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 100a;

A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 100b;

A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 100c;

A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 100d;

A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W or Y at the amino acid position 100e;

A, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 100f;

A, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 100g;

A, D, E, G, H, I, L, M, N, P, S, T or V at the amino acid position 100h;

A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 100i;

A, D, F, I, L, M, N, Q, S, T or V at the amino acid position 101;

A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W or Y at the amino acid position 102;

and/or

- (b) a light chain variable domain amino acid sequence comprising, at each of the following positions (all by Kabat numbering), one or more of the following amino acid residues indicated for that position:

A, D, F, G, H, I, K, L, M, N, Q, R, S, T, V, W or Y at the amino acid position 24;

A, G, N, P, S, T or V at the amino acid position 25;

A, D, E, F, G, I, K, L, M, N, Q, R, S, T or V at the amino acid position 26;

A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W or Y at the amino acid position 27;

A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 27a;
 A, I, L, M, P, T or V at the amino acid position 27b;
 A, E, F, H, I, K, L, M, N, P, Q, R, T, W or Y at the amino acid position 27c;
 A, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 27d;
 A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 27e;
 G, N, S or T at the amino acid position 28;
 A, F, G, H, K, L, M, N, Q, R, S, T, W or Y at the amino acid position 29;
 A, F, G, H, I, K, L, M, N, Q, R, V, W or Y at the amino acid position 30;
 I, L, Q, S, T or V at the amino acid position 31;
 F, W or Y at the amino acid position 32;
 A, F, H, L, M, Q or V at the amino acid position 33;
 A, H or S at the amino acid position 34;
 I, K, L, M or R at the amino acid position 50;
 A, E, I, K, L, M, Q, R, S, T or V at the amino acid position 51;
 A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W or Y at the amino acid position 52;
 A, E, F, G, H, K, L, M, N, P, Q, R, S, V, W or Y at the amino acid position 53;
 A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 54;
 A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V or Y at the amino acid position 55;
 A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W or Y at the amino acid position 56;
 A, G, K, S or Y at the amino acid position 89;
 Q at the amino acid position 90;
 G at the amino acid position 91;
 A, D, H, K, N, Q, R, S or T at the amino acid position 92;
 A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W or Y at the amino acid position 93;
 A, D, H, I, M, N, P, Q, R, S, T or V at the amino acid position 94;
 P at the amino acid position 95;
 F or Y at the amino acid position 96; and
 A, D, E, G, H, I, K, L, M, N, Q, R, S, T or V at the amino acid position 97.

65. The antigen-binding molecule of any one of claims 1 to 54, wherein the antigen-binding molecule has at least one characteristic selected from the group consisting of (1) ~~or to~~ (23) below:
- (1) the antigen-binding molecule ~~does not~~ bind to either of CD3 ~~and/or~~ CD137 each expressed on a different cell, ~~at the same time.~~
 - ~~(2) the antigen-binding molecule has an agonistic activity against CD137; and~~

- (23) the antigen-binding molecule has equivalent or 10-fold, 20-fold, 50-fold, 100-fold lower KD value for binding to human CD137, as compared to a reference antibody comprising a VH sequence of SEQ ID NO: 1 and a VL sequence of SEQ ID NO: 57, wherein the KD value is preferably measured by SPR at the following condition: 37 degrees C, pH 7.4, 20 mM ACES, 150 mM NaCl, 0.05% Tween 20, 0.005% NaN3; the antigen-binding molecule is immobilized on a CM4 sensor chip, the antigen serves as analyte.
- 76 The antigen-binding molecule of any one of claims 1 to 5, which further comprises an antibody variable region that is capable of binding to a third antigen different from CD3 and CD137.
87. The antigen-binding molecule of claim 67, wherein the third antigen is a molecule specifically expressed in a cancer tissue.
9. The antigen-binding molecule of claim 7 or 8, wherein the antigen-binding molecule has at least one characteristic selected from the group consisting of (1) to (5) below:
- (1) the antigen-binding molecule induces CD3 activation of a T cell against a cell expressing the molecule of the third antigen, but does not induce CD3 activation of a T cell against a cell expressing CD137;
- (2) the antigen-binding molecule induces cytotoxicity of a T cell against a cell expressing the molecule of the third antigen, but does not induce cytotoxicity of a T cell against a cell expressing CD137;
- (3) the antigen-binding molecule does not induce a cytokine release from PBMC in the absence of a cell expressing the molecule of the third antigen;
- (4) the antigen-binding molecule induces equivalent to or 2-fold, 5-fold, 10-fold, 20-fold or 100-fold greater CD137 activation and/or cytotoxicity of a T cell against a cell expressing the molecule of the third antigen, as compared to a reference antibody comprising a VH sequence of SEQ ID NO: 1 and a VL sequence of SEQ ID NO: 57; and/or
- (5) the antigen-binding molecule induces 2-fold, 5-fold, 10-fold, 20-fold or 100-fold greater cytotoxicity of a T cell against a cell expressing the molecule of the third antigen while does not induce a cytokine (IL-6) release from PBMC, as compared to a reference bispecific antibody targeting the third antigen and CD3.
108. The antigen-binding molecule of any one of claims 1 to 97, further comprising an antibody Fc region.
119. The antigen-binding molecule of claim 108, wherein the Fc region is an Fc region having reduced binding activity against Fc gamma R as compared with the Fc region of a naturally occurring human IgG1 antibody.
1240. An isolated polynucleotide comprising a nucleotide sequence that encodes the antigen-binding molecule of any one of claims 1 to 119.
1344. An expression vector comprising the polynucleotide according to claim 1240.

1412. A host cell transformed or transfected with the polynucleotide according to claim 1240 or the expression vector according to claim 1341.
1513. A method of producing a multispecific antigen-binding molecule or a multispecific antibody, comprising culturing the host cell of claim 1412.
1614. A multispecific antigen-binding molecule or a multispecific antibody as obtainable by the method of claim 1513.
1715. A method of obtaining or screening for an antibody variable region that is capable of binding to CD3 and CD137, ~~andbut does not~~ bind to either of CD3 ~~andor~~ CD137 ~~at the same time~~, comprising:
- (a) providing a library comprising a plurality of antibody variable region,
 - (b) contacting the library provided in step (a) with either CD3 or CD137 as a first antigen and collecting antibody variable regions bound to the first antigen,
 - (c) contacting the antibody variable regions collected in step (b) with a second antigen out of CD3 and CD137 and collecting antibody variable regions bound to the second antigen, and
 - (d) selecting an antibody variable region which:
 - (1) has an agonistic activity against CD137 and binds to CD137 with an equilibrium dissociation constant (KD) of less than about 5×10^{-6} M or between 5×10^{-6} M and 3×10^{-8} M, preferably as measured by SPR at the following condition: 37 degrees C, pH 7.4, 20 mM ACES, 150 mM NaCl, 0.05% Tween 20, 0.005% NaN₃; the antigen-binding molecule is immobilized on a CM4 sensor chip, the antigen serves as analyte; and/or
 - (2) binds to CD3 with an equilibrium dissociation constant (KD) of between 2×10^{-6} M and 1×10^{-8} M, preferably as measured by SPR at the following condition: 25 degrees C, pH 7.4, 20 mM ACES, 150 mM NaCl, 0.05% Tween 20, 0.005% NaN₃; the antigen-binding molecule is immobilized on a CM4 sensor chip, the antigen serves as analyte.
4618. A pharmaceutical composition comprising the antigen-binding molecule according to any one of claims 1 to 119, or the multispecific antigen-binding molecule or multispecific antibody according to claim 1614, and a pharmaceutically acceptable carrier.
19. The pharmaceutical composition of claim 18 or the antigen-binding molecule of any one of claims 1 to 11 for use in the treatment of cancer.
20. Use of the pharmaceutical composition of claim 18 or the antigen-binding molecule of any one of claims 1 to 11 in the manufacture of a medicament for treating cancer.

21. Use of the pharmaceutical composition of claim 18 or the antigen-binding molecule of any one of claims 1 to 11 in the manufacture of a medicament for preventing, treating or inhibiting cancer in a mammalian subject suffering from cancer.

22. Use of the pharmaceutical composition of claim 18 or the antigen-binding molecule of any one of claims 1 to 11 in the manufacture of a medicament for inducing cytotoxicity, preferably T-cell dependent cytotoxicity in a mammalian subject suffering from cancer.

23. Use of the pharmaceutical composition of claim 18 or the antigen-binding molecule of any one of claims 1 to 11 for reducing or killing cancer cell in a mammalian subject suffering from cancer.

24. Use of the pharmaceutical composition of claim 18 or the antigen-binding of any one of claims 1 to 11 for extending lifespan or survival rate of a cancer in a mammalian subject suffering from cancer.