

[NPL 8] Clynes *et al.*, Nat Med (2000) 6: 443-446

[NPL 9] Lazar *et al.*, Proc Natl Acad Sci U S A (2006) 103: 4005-4010

[NPL 10] Chu *et al.*, Mol Immunol (2008) 45: 3926-3933

[NPL 11] Radaev *et al.*, J Biol Chem (2001) 276: 16469-16477

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[Summary of Invention]

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In one aspect, the present disclosure refers to anti-CTLA-4 antibody having a CTLA-4 binding activity that is dependent on the concentration of an adenosine-containing compound, wherein the antibody has at least one feature selected from (a) to (i) below: (a) a binding activity in the presence of 100 μ M adenosine-containing compound is two times or more higher than that in the absence of the adenosine-containing compound; (b) KD value in the presence of 100 μ M adenosine-containing compound is 5×10^{-7} M or less; (c) KD value in the absence of the adenosine-containing compound is 1×10^{-6} M or more; (d) forming a ternary complex with the adenosine-containing compound and CTLA-4; (e) binding to a region from the amino acid at position 97 to the amino acid at position 106 of the human CTLA-4 (extracellular domain, SEQ ID NO: 28); (f) competing with ABAM004 (VH, SEQ ID NO: 10; and VL, SEQ ID NO: 11) for binding to CTLA-4; (g) binding to the same epitope as that of ABAM004 (VH, SEQ ID NO: 10; and VL, SEQ ID NO: 11); (h) showing cytotoxic activity against a CTLA-4-expressing cell; and (i) binding to human- and mouse-derived CTLA-4.

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In another aspect, the present disclosure refers to an anti-CTLA-4 antibody, which comprises: (1) a VH sequence as set forth in SEQ ID NO: 98 and a VL sequence as set forth in SEQ ID NO: 99; (2) a VH sequence as set forth in SEQ ID NO: 83 and a VL sequence as set forth in SEQ ID NO: 97; (3) a VH sequence as set forth in SEQ ID NO: 86 and a VL sequence as set forth in SEQ ID NO: 134; (4) a VH sequence as set forth in SEQ ID NO: 136 and a VL sequence as set forth in SEQ ID NO: 95; (5) a VH sequence as set forth in SEQ ID NO: 140 and a VL sequence as set forth in SEQ ID NO: 146; (6) a VH sequence as set forth in SEQ ID NO: 141 and a VL sequence as set forth in SEQ ID NO: 146; (7) a VH sequence as set forth in SEQ ID NO: 140 and a VL sequence as set forth in SEQ ID NO: 147; (8) a VH sequence as set forth in SEQ ID NO: 141 and a VL sequence as set forth in SEQ ID NO: 147; (9) a VH sequence as set forth in SEQ ID NO: 136 and a VL sequence as set forth in SEQ ID NO: 149; (10) a first variable region comprising a VH sequence as set forth in SEQ ID NO: 140 and a VL sequence as set forth in SEQ ID NO: 146 and a second variable region comprising a VH sequence as set forth in SEQ ID NO: 141 and a VL sequence as set forth in SEQ ID NO: 146; or (11) a first variable region comprising a VH sequence as set forth in SEQ ID NO: 140 and a VL sequence as set forth in SEQ ID NO: 147 and a second variable region comprising a VH sequence as set forth in SEQ ID NO: 141 and a VL sequence as set forth in SEQ ID NO: 147.

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In another aspect, the present disclosure refers to a pharmaceutical formulation comprising the antibody as disclosed herein and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure refers to the use of the pharmaceutical formulation of as disclosed herein in the manufacture of a medicament for treating a tumor.

In yet another aspect, the present disclosure refers to a polypeptide, which comprises a variant Fc region comprising amino acid alterations in a parent Fc region, wherein the parent Fc region is composed of two polypeptide chains, and wherein the variant Fc region comprises amino acid alterations at the following positions: (i) positions 234, 235, 236, 239, 250, 268, 270, 298, 307, and 326 according to EU numbering in a first polypeptide of the parent Fc region; and (ii) positions 236, 250, 270, 298, 307, 326, and 334 according to EU numbering in a second polypeptide of the parent Fc region.

[Technical Problem]

[0005]

The present invention provides anti-CTLA-4 antibodies and methods of using the antibodies. The present invention also provides polypeptides comprising a variant Fc region and methods of producing the polypeptides.

[Solution to Problem]

[0006]

More specifically, the present invention provides [1] to [47] below.

[1] An anti-CTLA-4 antibody having a CTLA-4 binding activity that is dependent on the concentration of an adenosine-containing compound, wherein the antibody has at least one feature selected from (a) to (i) below:

(a) a binding activity in the presence of 100 μ M adenosine-containing compound is twice or more higher than that in the absence of the adenosine-containing compound;

(b) K_D value in the presence of 100 μ M adenosine-containing compound is 5×10^{-7} M or less;

(c) K_D value in the absence of the adenosine-containing compound is 1×10^{-6} M or more;

(d) forming a ternary complex with the adenosine-containing compound and CTLA-4;

(e) binding to a region from the amino acid at position 97 to the amino acid at position 106 of the human CTLA-4 (extracellular domain, SEQ ID NO: 28);

(f) competing with ABAM004 (VH, SEQ ID NO: 10; and VL, SEQ ID NO: 11) for binding to CTLA-4;

(g) binding to the same epitope as that bound by ABAM004 (VH, SEQ ID NO: 10; and VL, SEQ ID NO: 11);

5 (h) showing cytotoxic activity against a CTLA-4-expressing cell; and

(i) binding to human- and mouse-derived CTLA-4.

[2] The antibody of [1], wherein the antibody is a monoclonal antibody.

[3] The antibody of [1] or [2], wherein the antibody is a human antibody, humanized antibody, or chimeric antibody.

[CLAIMS]

1. An anti-CTLA-4 antibody having a CTLA-4 binding activity that is dependent on the concentration of an adenosine-containing compound, wherein the antibody has at least one
5 feature selected from (a) to (i) below:
 - (a) a binding activity in the presence of 100 μ M adenosine-containing compound is two times or more higher than that in the absence of the adenosine-containing compound;
 - (b) K_D value in the presence of 100 μ M adenosine-containing compound is 5×10^{-7} M or less;
 - 10 (c) K_D value in the absence of the adenosine-containing compound is 1×10^{-6} M or more;
 - (d) forming a ternary complex with the adenosine-containing compound and CTLA-4;
 - (e) binding to a region from the amino acid at position 97 to the amino acid at position 106 of the human CTLA-4 (extracellular domain, SEQ ID NO: 28);
 - 15 (f) competing with ABAM004 (VH, SEQ ID NO: 10; and VL, SEQ ID NO: 11) for binding to CTLA-4;
 - (g) binding to the same epitope as that of ABAM004 (VH, SEQ ID NO: 10; and VL, SEQ ID NO: 11);
 - (h) showing cytotoxic activity against a CTLA-4-expressing cell; and
 - 20 (i) binding to human- and mouse-derived CTLA-4.

2. The antibody of claim 1, wherein the antibody comprises: (a) HVR-H1 (SEQ ID NO: 223) comprising the amino acid sequence SX_1TMN , wherein X_1 is H, A, R, or K; (b) HVR-H2 (SEQ ID NO: 224) comprising the amino acid sequence $SISX_1X_2SX_3YIYYAX_4SVX_5G$, wherein X_1 is
25 S or T, X_2 is R or Q, X_3 is G or H, X_4 is D, E, or R, and X_5 is K or R; and (c) HVR-H3 (SEQ ID NO: 225) comprising the amino acid sequence $YGX_1REDMLWVFDY$, wherein X_1 is K or A.

3. The antibody of claim 2, which further comprises: (a) HVR-L1 (SEQ ID NO: 226) comprising the amino acid sequence $X_1GX_2STX_3VGDYX_4X_5VX_6$, wherein X_1 is T, D, Q, or E,
30 X_2 is T or P, X_3 is D or G, X_4 is N or T, X_5 is Y or W, and X_6 is S or H; (b) HVR-L2 (SEQ ID NO: 227) comprising the amino acid sequence $X_1TX_2X_3KPX_4$, wherein X_1 is E, F, or Y, X_2 is S or I, X_3 is K or S, and X_4 is S, E, or K; and (c) HVR-L3 (SEQ ID NO: 228) comprising the amino acid sequence $X_1TYAAPLGPX_2$, wherein X_1 is S or Q and X_2 is M or T.

- 35 4. The antibody of claim 3, which comprises:
(1) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 100; (b) HVR-H2

acid sequence of SEQ ID NO: 102; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 129; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 133;

(9) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 107; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 111; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 152; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 129; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 133; or

(10) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 107; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 109; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 102; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 130; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 133.

45. The antibody of claim 1, which comprises: (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 83 to 86, 98, and 135 to 141; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 88 to 95, 97, 99, 134, and 144 to 149; or (c) a VH sequence having the amino acid sequence of any one of SEQ ID NOs: 83 to 86, 98, and 135 to 141 and a VL sequence having the amino acid sequence of any one of SEQ ID NOs: 88 to 95, 97, 99, 134, and 144 to 149.

6. An anti-CTLA-4 antibody, which comprises:

(1) a VH sequence as set forth in SEQ ID NO: 98 and a VL sequence as set forth in SEQ ID NO: 99;

(2) a VH sequence as set forth in SEQ ID NO: 83 and a VL sequence as set forth in SEQ ID NO: 97;

(3) a VH sequence as set forth in SEQ ID NO: 86 and a VL sequence as set forth in SEQ ID NO: 134;

(4) a VH sequence as set forth in SEQ ID NO: 136 and a VL sequence as set forth in SEQ ID NO: 95;

(5) a VH sequence as set forth in SEQ ID NO: 140 and a VL sequence as set forth in SEQ ID NO: 146;

(6) a VH sequence as set forth in SEQ ID NO: 141 and a VL sequence as set forth in SEQ ID NO: 146;

(7) a VH sequence as set forth in SEQ ID NO: 140 and a VL sequence as set forth in SEQ

ID NO: 147;

(8) a VH sequence as set forth in SEQ ID NO: 141 and a VL sequence as set forth in SEQ ID NO: 147;

(9) a VH sequence as set forth in SEQ ID NO: 136 and a VL sequence as set forth in SEQ ID NO: 149;

(10) a first variable region comprising a VH sequence as set forth in SEQ ID NO: 140 and a VL sequence as set forth in SEQ ID NO: 146 and a second variable region comprising a VH sequence as set forth in SEQ ID NO: 141 and a VL sequence as set forth in SEQ ID NO: 146; or

(11) a first variable region comprising a VH sequence as set forth in SEQ ID NO: 140 and a VL sequence as set forth in SEQ ID NO: 147 and a second variable region comprising a VH sequence as set forth in SEQ ID NO: 141 and a VL sequence as set forth in SEQ ID NO: 147.

57. A pharmaceutical formulation comprising the antibody of any one of claims 1 to 46 and a pharmaceutically acceptable carrier.

68. ~~Use of the~~The pharmaceutical formulation of claim 57 ~~in the manufacture of a medicament for, wherein the pharmaceutical formulation is for use in~~ treating a tumor.

79. The ~~pharmaceutical formulation~~use of claim 68, wherein immunity in a tumor tissue is to be activated with a dose lower than that in a non-tumor tissue.

810. A polypeptide, which comprises a variant Fc region comprising amino acid alterations in a parent Fc region, wherein the parent Fc region is composed of two polypeptide chains, and wherein the variant Fc region comprises amino acid alterations at the following positions:

(i) positions 234, 235, 236, 239, 250, 268, 270, 298, 307, and 326 according to EU numbering in a first polypeptide of the parent Fc region; and

(ii) positions 236, 250, 270, 298, 307, 326, and 334 according to EU numbering in a second polypeptide of the parent Fc region.

911. The polypeptide of claim 810, wherein the binding activity to at least one Fcγ receptor selected from the group consisting of FcγRIa, FcγRIIa, FcγRIIb, and FcγRIIIa is enhanced in the variant Fc region compared to that in the parent Fc region.

1012. The polypeptide of claim 8-10 or 911, wherein the selectivity between an activating Fcγ receptor and an inhibitory Fcγ receptor is improved in the variant Fc region compared to that in

the parent Fc region.

[Title of Invention] ANTI-CTLA-4 ANTIBODY AND USE THEREOF

[ABSTRACT]

5 The present disclosure provides anti-CTLA-4 antibodies and methods of producing and
using the antibodies. The present disclosure also provides nucleic acids encoding the anti-
CTLA-4 antibodies and host cells containing the nucleic acids. Furthermore, the present
disclosure provides polypeptides containing a variant Fc region and methods of producing and
using the polypeptides.

10 [no suitable figure]