

[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]

In one aspect, the present disclosure refers to an anti-CTLA-4 antibody comprising: (a) HVR-H1 (SEQ ID NO: 223) comprising the amino acid sequence SX₁TMN, wherein X₁ is H, A, R, or K; (b) HVR-H2 (SEQ ID NO: 224) comprising the amino acid sequence SISX₁X₂SX₃YIYYAX₄SVX₅G, wherein X₁ is S or T, X₂ is R or Q, X₃ is G or H, X₄ is D, E, or R, and X₅ is K or R; (c) HVR-H3 (SEQ ID NO: 225) comprising the amino acid sequence YGX₁REDMLWVFDY, wherein X₁ is K or A; (d) HVR-L1 (SEQ ID NO: 226) comprising the amino acid sequence X₁GX₂STX₃VGDYX₄X₅VX₆, wherein X₁ is T, D, Q, or E, X₂ is T or P, X₃ is D or G, X₄ is N or T, X₅ is Y or W, and X₆ is S or H; (e) HVR-L2 (SEQ ID NO: 227) comprising the amino acid sequence X₁TX₂X₃KPX₄, wherein X₁ is E, F, or Y, X₂ is S or I, X₃ is K or S, and X₄ is S, E, or K; and (f) HVR-L3 (SEQ ID NO: 228) comprising the amino acid sequence X₁TYAAPLGPX₂, wherein X₁ is S or Q and X₂ is M or T.

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

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 as disclosed herein in the manufacture of a medicament for treating a tumor.

[Technical Problem]

5 [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.

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

20 (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
25 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);

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

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

30 [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 comprising:
 - (a) HVR-H1 (SEQ ID NO: 223) comprising the amino acid sequence SX_1TMN , wherein X_1 is H, A, R, or K;
 - 5 (b) HVR-H2 (SEQ ID NO: 224) comprising the amino acid sequence $SISX_1X_2SX_3YIYYAX_4SVX_5G$, wherein X_1 is 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;
 - (c) HVR-H3 (SEQ ID NO: 225) comprising the amino acid sequence $YGX_1REDMLWVFDY$, wherein X_1 is K or A;
 - 10 (d) HVR-L1 (SEQ ID NO: 226) comprising the amino acid sequence $X_1GX_2STX_3VG DYX_4X_5VX_6$, wherein X_1 is T, D, Q, or E, 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;
 - (e) 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
 - 15 (f) HVR-L3 (SEQ ID NO: 228) comprising the amino acid sequence $X_1TYAAPLG PX_2$, wherein X_1 is S or Q and X_2 is M or T.
2. The antibody of claim 1, which comprises:
 - (1)
 - (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 100;
 - 20 (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 101;
 - (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: 113;
 - (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 114; and
 - (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 115,
 - 25 (2)
 - (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 100;
 - (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 104;
 - (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: 116;
 - (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117; and
 - 30 (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 115,
 - (3)
 - (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 105;
 - (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 106;
 - (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: 122;
 - 35 (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117; and
 - (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 133,

- (4) (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: 108;
 (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: 121;
 5 (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 123; and
 (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 153,
- (5) (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: 110;
 (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 102;
 10 (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122;
 (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117; and
 (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 133,
- (6) (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: 112;
 15 (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: 128;
 (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117; and
 (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 133,
- (7) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 107;
 20 (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: 128;
 (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117; and
 (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 133,
- 25 (8) (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: 112;
 (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: 129;
 (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117; and
 30 (f) HVR-L3 comprising the amino acid sequence of 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;
 35 (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117; and
 (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 133.

3. 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;

5 (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.

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4. An anti-CTLA-4 antibody comprising:

(1) the VH sequence of SEQ ID NO: 98 and the VL sequence of SEQ ID NO: 99,

(2) the VH sequence of SEQ ID NO: 83 and the VL sequence of SEQ ID NO: 97,

(3) the VH sequence of SEQ ID NO: 86 and the VL sequence of SEQ ID NO: 134,

15 (4) the VH sequence of SEQ ID NO: 136 and the VL sequence of SEQ ID NO: 95,

(5) the VH sequence of SEQ ID NO: 140 and the VL sequence of SEQ ID NO: 146,

(6) the VH sequence of SEQ ID NO: 141 and the VL sequence of SEQ ID NO: 146,

(7) the VH sequence of SEQ ID NO: 140 and the VL sequence of SEQ ID NO: 147,

(8) the VH sequence of SEQ ID NO: 141 and the VL sequence of SEQ ID NO: 147,

20 (9) the VH sequence of SEQ ID NO: 136 and the VL sequence of SEQ ID NO: 149,

(10) a first variable region comprising the VH sequence of SEQ ID NO: 140 and the VL sequence of SEQ ID NO: 146, and a second variable region comprising the VH sequence of SEQ ID NO: 141 and the VL sequence of SEQ ID NO: 146, or

(11) a first variable region comprising the VH sequence of SEQ ID NO: 140 and the VL sequence of SEQ ID NO: 147, and a second variable region comprising the VH sequence of SEQ ID NO: 141 and the VL sequence of SEQ ID NO: 147.

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5. A pharmaceutical formulation comprising the antibody of any one of claims 1 to 4 and a pharmaceutically acceptable carrier.

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6. Use of the pharmaceutical formulation of claim 5 in the manufacture of a medicament for treating a tumor.

7. The use of claim 6, wherein immunity in a tumor tissue is to be activated with a dose lower than that in a non-tumor tissue.

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