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[Summary of Invention]

In one aspect, the present disclosure provides an anti-CTLA-4 antibody comprising:

- 20 (A) a variable region comprising:
 - (1) (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: 128,
 - 25 (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;
 - (2) (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,
 - 30 (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;
 - (3) (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,
 - 35 (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
- (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 133; or
- (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: 111,
- 5 (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 the amino acid sequence of SEQ ID NO: 133; and
- (B) a variant Fc region comprising:
 - 10 (i) amino acid alterations to Phe at position 234, to Gln at position 235, to Trp at position 236, to Met at position 239, to Asp at position 268, to Glu at position 270, to Ala at position 298, and to Met at position 330 according to EU numbering in the first polypeptide of a parent Fc region composed of two polypeptide chains; and
 - (ii) amino acid alterations to Glu at position 270, to Ala at position 298, to Asp at
 - 15 position 326, to Met at position 330, and to Glu at position 334 according to EU numbering in the second polypeptide of the parent Fc region.

In another aspect, the present disclosure provides an anti-CTLA-4 antibody comprising:

- (1) the first H chain polypeptide of SEQ ID NO: 335, the second H chain
- 20 polypeptide of SEQ ID NO: 336, and the L chain polypeptides of SEQ ID NO: 161, or
- (2) the first H chain polypeptide of SEQ ID NO: 337, the second H chain polypeptide of SEQ ID NO: 338, and the L chain polypeptides of SEQ ID NO: 161.

In another aspect, the present disclosure provides pharmaceutical formulation comprising the anti-CTLA-4 antibody of the disclosure and a pharmaceutically

25 acceptable carrier.

[Technical Problem]

[0010]

The present invention provides anti-CTLA-4 antibodies and methods of using

30 the antibodies. The present invention also provides polypeptides comprising a variant Fc region and methods of producing the polypeptides.

[Solution to Problem]

[0011]

More specifically, the present invention provides [1] to [26] below.

35 [1] An anti-CTLA-4 antibody comprising:

(A) a variable region having a CTLA-4 binding activity that is dependent on the concentration of an adenosine-containing compound; and
(B) a variant Fc region comprising a plurality of amino acid alterations in a parent Fc region,

- 5 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, 268, 270, 298, and 330 according to EU numbering in the first polypeptide of the parent Fc region; and

[CLAIMS]

[Claim 1]

An anti-CTLA-4 antibody comprising:

(A) a variable region comprising:

- 5 (1) (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: 128,
 (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117, and
 10 (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 133;
- (2) (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: 128,
 15 (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) (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,
 20 (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 the amino acid sequence of SEQ ID NO: 133; or
- (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: 111,
 25 (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 the amino acid sequence of SEQ ID NO: 133; and

(B) a variant Fc region comprising:

- 30 (i) amino acid alterations to Phe at position 234, to Gln at position 235, to Trp at
 position 236, to Met at position 239, to Asp at position 268, to Glu at position 270, to
 Ala at position 298, and to Met at position 330 according to EU numbering in the first
 polypeptide of a parent Fc region composed of two polypeptide chains; and
- (ii) amino acid alterations to Glu at position 270, to Ala at position 298, to Asp at
 35 position 326, to Met at position 330, and to Glu at position 334 according to EU
 numbering in the second polypeptide of the parent Fc region.

[Claim 2]

The anti-CTLA-4 antibody of claim 1, which comprises: (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 140 or 141; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 146 or 147; or (c) a VH sequence having the amino acid sequence of SEQ ID NO: 140 or 141 and a VL sequence having the amino acid sequence of SEQ ID NO: 146 or 147.

10 [Claim 3]

The anti-CTLA-4 antibody of claim 1, which comprises:

- (1) the VH sequence of SEQ ID NO: 140 and the VL sequence of SEQ ID NO: 146;
- (2) the VH sequence of SEQ ID NO: 141 and the VL sequence of SEQ ID NO: 146;
- (3) the VH sequence of SEQ ID NO: 140 and the VL sequence of SEQ ID NO: 147;
- 15 (4) the VH sequence of SEQ ID NO: 141 and the VL sequence of SEQ ID NO: 147;
- (5) the first variable region comprising the VH sequence of SEQ ID NO: 140 and the VL sequence of SEQ ID NO: 146, and the second variable region comprising the VH sequence of SEQ ID NO: 141 and the VL sequence of SEQ ID NO: 146; or
- (6) the first variable region comprising the VH sequence of SEQ ID NO: 140 and the VL sequence of SEQ ID NO: 147, and the second variable region comprising the VH sequence of SEQ ID NO: 141 and the VL sequence of SEQ ID NO: 147.

[Claim 4]

The anti-CTLA-4 antibody of claim 1, wherein the variant Fc region further comprises:

- (i) amino acid alterations to Val at position 250 and to Pro at position 307 according to EU numbering in the first polypeptide of the parent Fc region; and
- (ii) amino acid alterations to Val at position 250 and to Pro at position 307 according to EU numbering in the second polypeptide of the parent Fc region.

[Claim 5]

The anti-CTLA-4 antibody of claim 1, wherein the variant Fc region further comprises any of the amino acid alterations of (a) to (f) below:

- (a) an amino acid alteration to Lys at position 356 according to EU numbering in the first polypeptide of the parent Fc region and an amino acid alteration to Glu at position 439 according to EU numbering in the second polypeptide of the parent Fc region,
- (b) an amino acid alteration to Glu at position 439 according to EU numbering in the first polypeptide of the parent Fc region and an amino acid alteration to Lys at position 356 according to EU numbering in the second polypeptide of the parent Fc region,
- (c) an amino acid alteration to Trp at position 366 according to EU numbering in the first polypeptide of the parent Fc region and amino acid alterations to Ser at position 366, to Ala at position 368, and to Val at position 407 according to EU numbering in the second polypeptide of the parent Fc region,
- (d) amino acid alterations to Ser at position 366, to Ala at position 368, and to Val at position 407 according to EU numbering in the first polypeptide of the parent Fc region and an amino acid alteration to Trp at position 366 according to EU numbering in the second polypeptide of the parent Fc region,
- (e) amino acid alterations to Cys at position 349 and to Trp at position 366 according to EU numbering in the first polypeptide of the parent Fc region and amino acid alterations to Cys at position 356, to Ser at position 366, to Ala at position 368, and to Val at position 407 according to EU numbering in the second polypeptide of the parent Fc region, and
- (f) amino acid alterations to Cys at position 356, to Ser at position 366, to Ala at position 368, and to Val at position 407 according to EU numbering in the first polypeptide of the parent Fc region and amino acid alterations to Cys at position 349 and to Trp at position 366 according to EU numbering in the second polypeptide of the parent Fc region.

[Claim 6]

The anti-CTLA-4 antibody of claim 1, which comprises a heavy chain constant region comprising the variant Fc region.

[Claim 7]

The anti-CTLA-4 antibody of claim 1, wherein the heavy chain constant region comprises

(1) the first polypeptide of SEQ ID NO: 358 and the second polypeptide of SEQ ID NO: 359, or

(2) the first polypeptide of SEQ ID NO: 360 and the second polypeptide of SEQ ID NO: 361.

[Claim 8]

An anti-CTLA-4 antibody comprising:

- 5 (1) the first H chain polypeptide of SEQ ID NO: 335, the second H chain polypeptide of SEQ ID NO: 336, and the L chain polypeptides of SEQ ID NO: 161, or
- (2) the first H chain polypeptide of SEQ ID NO: 337, the second H chain polypeptide of SEQ ID NO: 338, and the L chain polypeptides of SEQ ID NO: 161.

[Claim 9]

- 10 A pharmaceutical formulation comprising the anti-CTLA-4 antibody of any one of claims 1 to 8 and a pharmaceutically acceptable carrier.