

Alzheimer's disease; and diseases with inflammatory or allergic components such as graft versus host disease, host versus graft disease, allergies, autoimmune diseases and other inflammatory diseases.

[0011] Though antibodies against CTLA4 are known and commercially available, it is desirable to find improved anti-CTLA4 antibodies that are suitable for cancer therapy with reduced or minimal side effects. The present invention provides anti-CTLA4 antibodies or antibody fragments that are suitable for therapeutic and diagnostic use, especially for diagnosis and treatment of cancers. Some of these anti-CTLA4 antibodies or antibody fragments may have a higher binding affinity to CTLA4 in a tumor in comparison with CTLA4 present in normal tissue. These anti-CTLA4 antibodies or antibody fragments typically have at least comparable efficacy to known anti-CTLA4 antibodies or antibody fragments. In addition, the present anti-CTLA4 antibodies or antibody fragments may exhibit reduced side effects in comparison with monoclonal anti-CTLA4 antibodies known in the art. These advantages may provide a more selective treatment of the CTLA4 in a tumor and may permit use of higher dosages of these anti-CTLA4 antibodies or antibody fragments as a result of the selectivity for CTLA4 in a tumor, whereby more effective therapeutic treatments can be realized without a corresponding increase in undesirable side effects.

SUMMARY OF THE DISCLOSURE

[0012] In one example, the present disclosure provides isolated heavy chain variable region polypeptides that specifically bind to the CTLA4 protein. These polypeptides include three complementarity determining regions having the H1, H2, and H3 sequences, wherein:

the H1 sequence is GFTFSHYTMH (SEQ ID NO: 1);

the H2 sequence is FIX₁YX₂GNX₃KX₄X₅AX₆SX₇KG (SEQ ID NO: 2); and

the H3 sequence is TGWLGPFDX₈ (SEQ ID NO: 3);

wherein X₁ is S or D; X₂ is D, H or I, X₃ is N or Y; X₄ is Y or I; X₅ is Y or E; X₆ is D or K; X₇ is V or M; and X₈ is Y or I.

[0013] In another example, the present disclosure includes a product formed by a combination of any of the above-described isolated heavy chain variable region polypeptides with an isolated light chain variable region polypeptide selected from isolated light chain variable region polypeptides that include three complementarity determining regions having the L1, L2, and L3 sequences, wherein:

the L1 sequence is RX₉SQX₁₀X₁₁GSSYLA (SEQ ID NO: 4);

the L2 sequence is GAFSRATGX₁₂ (SEQ ID NO: 5); and

the L3 sequence is QQDGSSPWT (SEQ ID NO: 6),
wherein X₉ is A or I; X₁₀ is Y, S or H; X₁₁ is V or G; X₁₂ is V or I.

[0013a] In one aspect, the present disclosure refers to an isolated polypeptide that specifically binds to CTLA4 protein, said polypeptide comprising:

a heavy chain variable region including three complementarity determining regions, said regions having H1, H2, and H3 sequences, wherein:

(a) the H1 sequence is GFTFSHYTMH (SEQ ID NO: 1);

(b) the H2 sequence is FIX₁YX₂GNX₃KX₄X₅AX₆SX₇KG (SEQ ID NO: 2); and

(c) the H3 sequence is TGWLGPFDX₈ (SEQ ID NO: 3),

wherein X₁ is S or D; X₂ is D, H or I; X₃ is N or Y; X₄ is Y or I; X₅ is Y or E; X₆ is D or K; X₇ is V or M; and X₈ is Y or I; and

a light chain variable region including three complementarity determining regions having L1, L2, and L3 sequences, wherein:

(a) the L1 sequence is RX₉SQX₁₀X₁₁GSSYLA (SEQ ID NO: 4);

(b) the L2 sequence is GAFSRATGX₁₂ (SEQ ID NO: 5); and

(c) the L3 sequence is QQDGSSPWT (SEQ ID NO: 6),

wherein X₉ is A or I; X₁₀ is Y, S or H; X₁₁ is V or G; X₁₂ is V or I.

[0014] In each of the previous examples, the H2 sequence may be selected from FIDYHGNNKYYADSVKG, FISYDGNNKIYADSVKG, FISYDGNNKYYADSVKG, FISYDGNYKYYADSVKG, FISYDGNYKYYAKSVKG, FISYHGNNKYEADSVKG, FISYHGNNKYYADSVKG, FISYIGNYKYYADSMKG, and FISYIGNYKYYADSVKG.

[0015] In each of the previous examples, the H3 sequence may be selected from TGWLGPFDY and TGWLGPFDI.

[0016] In each of the previous examples, the L1 sequence may be selected from RASQHVGGSSYLA, RASQSVGGSSYLA, RASQYGGSSYLA, RASQYVGGSSYLA, and RISQYVGGSSYLA.

[0017] In each of the previous examples, the L2 sequence may be selected from GAFSRATGI and GAFSRATGV.

[0018] In some examples, the isolated heavy chain variable region polypeptide may have a sequence selected from SEQ ID NOS: 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, and 38. In each of these examples, the isolated light chain variable region polypeptide may have a sequence selected from SEQ ID NOS: 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, and 37.

[0019] In one example, the antibody comprises a light chain variable region polypeptide and a heavy chain variable region polypeptide having a pair of sequences selected from the pairs: SEQ ID NOS: 7 and 8, SEQ ID NOS: 9 and 10, SEQ ID NOS: 11 and 12, SEQ ID NOS: 13 and 14, SEQ ID NOS: 15 and 16, SEQ ID NOS: 17 and 18, SEQ ID NOS: 19 and 20, SEQ ID NOS: 21 and 22, SEQ ID NOS: 23 and 24, SEQ ID NOS: 25 and 26, SEQ ID NOS: 27 and 28, SEQ ID NOS: 29 and 30, SEQ ID NOS: 31 and 32, SEQ ID NOS: 33 and 34, SEQ ID NOS: 35 and 36 and SEQ ID NOS: 37 and 38.

[0020] In yet another example, the present disclosure provides an anti-CTLA4 antibody or antibody fragment that includes any of the isolated heavy chain variable region polypeptides of the disclosure described above.

[0020a] In another aspect, the present disclosure refers to an anti-CTLA4 antibody or antibody fragment comprising the isolated polypeptide disclosed herein.

[0021] In yet another example, the present disclosure provides an anti-CTLA4 antibody or antibody fragment that includes a combination of any of the isolated heavy chain variable region polypeptides of the disclosure described above with any one of the isolated light chain variable region polypeptides of the disclosure described above.

[0022] In yet another example, the present disclosure provides an immunoconjugate that includes any of the antibody or antibody fragments of the disclosure described above. In another aspect, the present disclosure refers to an immunoconjugate comprising the antibody or antibody fragment disclosed herein. In another aspect, the present disclosure refers to an immunoconjugate comprising the antibody disclosed herein. In the

immunoconjugate, the antibody or antibody fragment may be conjugated to an agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.

[0023] In yet another example, the present disclosure provides a pharmaceutical composition that includes any of the polypeptides, the antibodies, the antibody fragments, and the immunoconjugates of the disclosure described above, together with a pharmaceutically acceptable carrier. In another aspect, the present disclosure refers to a pharmaceutical composition comprising: the polypeptide disclosed herein, the antibody or antibody fragment disclosed herein, or the immunoconjugate disclosed herein; a pharmaceutically acceptable carrier, and optionally, comprises a tonicity agent. In another aspect, the present disclosure refers to a pharmaceutical composition comprising: the antibody disclosed herein, or the immunoconjugate disclosed herein; and a pharmaceutically acceptable carrier, and optionally, further comprising a tonicity agent. A single dose of the pharmaceutical composition may include an amount of the polypeptide, the antibody, the antibody fragment, or the immunoconjugate of about 135 mg, about 235 mg, about 335 mg, about 435 mg, about 535 mg, about 635 mg, about 735 mg, about 835 mg, about 935 mg, about 1035 mg, about 1135 mg, about 1235 mg, or about 1387 mg.

[0024] A single dose of the pharmaceutical composition of may include an amount of the polypeptide, the antibody, the antibody fragment, or the immunoconjugate in a range of 135-1387 mg, 135 -235 mg, 235 -335 mg, 335 -435 mg, 435 -535 mg, 535 -635 mg, 635 -735 mg, 735 -835 mg, 835 -935 mg, 935 -1035 mg, 1035 -1135 mg, 1135 -1235 mg, or 1235 -1387 mg.

[0024a] In another aspect, the present disclosure refers to a single dose of the pharmaceutical composition disclosed herein, comprising an amount of the polypeptide disclosed herein, the antibody or antibody fragment disclosed herein, or the immunoconjugate disclosed herein in a range of 135 mg -235 mg, 235 mg -335 mg, 335 mg -435 mg, 435 mg -535 mg, 535 mg -635 mg, 635 mg -735 mg, 735 mg -835 mg, 835 mg -935 mg, 935 mg -1035 mg, 1035 mg -1135 mg, 1135 mg -1235 mg, or 1235 mg -1387 mg.

[0025] Each of the foregoing pharmaceutical compositions may further include an immune checkpoint inhibitor molecule that is different from the polypeptide or the antibody or antibody fragment. The immune checkpoint inhibitor molecule may be an antibody or antibody fragment against an immune checkpoint. The immune checkpoint may be selected from LAG3, TIM3, TIGIT, VISTA, BTLA, OX40, CD40, 4-1BB, PD-1, PD-L1, GITR, B7-H3, B7-H4, KIR, A2aR, CD27, CD70, DR3, and ICOS or the immune checkpoint may be PD-1 or PD-L1.

[0026] Each of the foregoing pharmaceutical compositions may further include an antibody or antibody fragment against an antigen selected from PD1, PD-L1, AXL, ROR2, CD3, HER2, B7-H3, ROR1, SFRP4 and a WNT protein. The WNT protein may be selected from WNT1, WNT2, WNT2B, WNT3, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9B, WNT10A, WNT10B, WNT11 and WNT16.

[0027] In yet another example, the present disclosure provides a kit for diagnosis or treatment including any of the polypeptides, the antibodies, the antibody fragments, or the immunoconjugates of the present disclosure described above.

[0028] In yet another aspect, the present disclosure refers to an anti-CTLA4 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises three complementarity determining regions having amino

acid sequences of SEQ ID NOS:39-41 and the light chain variable region comprises three complementarity determining regions having amino acid sequences of SEQ ID NOS:42-44. [0029] In the previous example, the heavy chain variable region may have an amino acid sequence of SEQ ID NO:8 and the light chain variable region may have an amino acid sequence of SEQ ID NO:7.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] FIG. 1 shows sequence alignments of exemplary heavy chain variable regions of anti-CTLA4 antibodies of the present invention.

[0031] FIG. 2 shows sequence alignments of exemplary light chain variable regions of anti-CTLA4 antibodies of the present invention.

[0032] FIG. 3A shows a comparison of the binding activity to human CTLA4 at pH 6.0 of two of the anti-CTLA4 antibodies of the present invention to Ipilimumab and an Ipilimumab analog (Ipi-analog), as measured by enzyme linked immunosorbent assay (ELISA).

[0033] FIG. 3B shows a comparison of the binding activity to human CTLA4 at pH 7.4 of the two anti-CTLA4 antibodies of the present invention of Fig. 3A to Ipilimumab and an Ipilimumab analog (Ipi-analog) as measured by ELISA.

[0034] FIG. 4A shows a comparison of the binding activity to cynomolgus CTLA4 at pH 6.0 of the two anti-CTLA4 antibodies of the present invention of Fig. 3A to Ipilimumab and an Ipilimumab analog (Ipi-analog) as measured by ELISA.

[0035] FIG. 4B shows a comparison of the binding activity to cynomolgus CTLA4 at pH 7.4 of the two anti-CTLA4 antibodies of the present invention of Fig. 3A to Ipilimumab and an Ipilimumab analog (Ipi-analog) as measured by ELISA.

[0036] FIG. 5 shows a comparison of the pH-dependent binding activity to human CTLA4 of the two anti-CTLA4 antibodies of the present invention of Fig. 3A to Ipilimumab and an Ipilimumab analog (Ipi-analog).

[0037] FIG. 6A shows a comparison of the half maximal effective concentration (EC_{50}) of, and the binding activity to human CTLA4 at pH 6.0 of the two anti-CTLA4 antibodies of the present invention of Fig. 3A to Ipilimumab and an Ipilimumab analog (Ipi-analog) as measured by fluorescence-activated cell sorting (FACS) using CHO cells.

[0038] FIG. 6B shows a comparison of the (EC_{50}) of, and the binding activity to human CTLA4 at pH 7.4 of the two anti-CTLA4 antibodies of the present invention of Fig. 3A to Ipilimumab and an Ipilimumab analog (Ipi-analog) as measured by FACS using CHO cells.

WHAT IS CLAIMED IS:

1. An isolated polypeptide that specifically binds to CTLA4 protein, said polypeptide comprising:

a heavy chain variable region including three complementarity determining regions, said regions having H1, H2, and H3 sequences, wherein:

(a) the H1 sequence is GFTFSHYTMH (SEQ ID NO: 1);

(b) the H2 sequence is FIX₁YX₂GNX₃KX₄X₅AX₆SX₇KG (SEQ ID NO: 2); and

(c) the H3 sequence is TGWLGPFDX₈ (SEQ ID NO: 3),

wherein X₁ is S or D; X₂ is D, H or I, X₃ is N or Y; X₄ is Y or I; X₅ is Y or E; X₆ is D or K; X₇ is V or M; and X₈ is Y or I; and

a light chain variable region including three complementarity determining regions having L1, L2, and L3 sequences, wherein:

(a) the L1 sequence is RX₉SQX₁₀X₁₁GSSYLA (SEQ ID NO: 4);

(b) the L2 sequence is GAFSRATGX₁₂ (SEQ ID NO: 5); and

(c) the L3 sequence is QQDGSSPWT (SEQ ID NO: 6),

wherein X₉ is A or I; X₁₀ is Y, S or H; X₁₁ is V or G; X₁₂ is V or I.

2. The polypeptide of claim 1, wherein the H2 sequence is selected from FIDYHGNNKYYADSVKG, FISYDGNNKIYADSVKG, FISYDGNNKYYADSVKG, FISYDGNYKYYADSVKG, FISYDGNYKYYAKSVKG, FISYHGNNKYEADSVKG, FISYHGNNKYYADSVKG, FISYIGNYKYYADSMKG, FISYIGNYKYYADSVKG, or wherein the H3 sequence is selected from TGWLGPFDY and TGWLGPFDI.

3. The polypeptide of claim 1, wherein the heavy chain variable region has an amino acid sequence selected from SEQ ID NOS: 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, or wherein the light chain variable region has an amino acid sequence selected from SEQ ID NOS: 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37.

4. The polypeptide of claim 1, wherein the L1 sequence is selected from RASQHVGGSSYLA, RASQSVGGSSYLA, RASQYGGSSYLA, RASQYVGGSSYLA, and RISQYVGGSSYLA, or wherein the L2 sequence is selected from GAFSRATGI and GAFSRATGV.

5. An anti-CTLA4 antibody or antibody fragment comprising the isolated polypeptide of any one of claims 1-4.
6. The antibody or antibody fragment of claim 5, wherein the antibody or antibody fragment has a higher binding affinity to CTLA4 protein at a value of a condition in a tumor microenvironment in comparison with a different value of a same condition that occurs in a non-tumor microenvironment.
7. The antibody or antibody fragment of claim 6, wherein the condition is pH, and the pH in the tumor microenvironment is in a range of from 5.0 to 6.8 and the pH in the non-tumor microenvironment is in a range of from 7.0 to 7.6.
8. The antibody or antibody fragment of any one of claims 5-7, wherein the antibody or antibody fragment has a ratio of binding affinity to the CTLA4 protein at a value of a condition in a tumor microenvironment to a binding affinity to the CTLA4 protein at a different value of the same condition in a non-tumor microenvironment of at least about 1.5:1, at least about 2:1, at least about 3:1, at least about 4:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 8:1, at least about 9:1, at least about 10:1, at least about 20:1, at least about 30:1, at least about 50:1, at least about 70:1, or at least about 100:1.
9. The antibody or antibody fragment of any one of claims 5-8, wherein the antibody or antibody fragment is a chimeric antibody, a multispecific antibody, or a humanized antibody.
10. An immunoconjugate comprising the antibody or antibody fragment of any one of claims 5-9.
11. The immunoconjugate of claim 10, wherein the immunoconjugate comprises at least one agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.
12. The immunoconjugate of claim 11, wherein the antibody or antibody fragment and the at least one agent are covalently bonded to a linker molecule, and the at least one agent is selected from maytansinoids, auristatins, dolastatins, calicheamicin, pyrrolobenzodiazepines, and anthracyclines.

13. A pharmaceutical composition comprising:
the polypeptide of any one of claims 1-4, the antibody or antibody fragment of any one of claims 5-9, or the immunoconjugate of any one of claims 10-12;
a pharmaceutically acceptable carrier, and optionally, comprises a tonicity agent.
14. A single dose of the pharmaceutical composition of claim 13, comprising an amount of the polypeptide of any one of claims 1-4, the antibody or antibody fragment of any one of claims 5-9, or the immunoconjugate of any one of claims 10-12 in a range of 135 mg -235 mg, 235 mg -335 mg, 335 mg -435 mg, 435 mg -535 mg, 535 mg -635 mg, 635 mg -735 mg, 735 mg -835 mg, 835 mg -935 mg, 935 mg -1035 mg, 1035 mg -1135 mg, 1135 mg -1235 mg, or 1235 mg -1387 mg .
15. The pharmaceutical composition of any one of claims 13-14, further comprising an immune checkpoint inhibitor molecule that is different from the polypeptide of any one of claims 1-4 and the antibody or antibody fragment of any one of claims 5-9.
16. The pharmaceutical composition of claim 15, wherein the immune checkpoint inhibitor molecule is an antibody or antibody fragment against an immune checkpoint.
17. The pharmaceutical composition of claim 16, wherein the immune checkpoint is selected from LAG3, TIM3, TIGIT, VISTA, BTLA, OX40, CD40, 4-1BB, PD-1, PD-L1, GITR, B7-H3, B7-H4, KIR, A2aR, CD27, CD70, DR3, and ICOS, or the immune checkpoint is PD-1 or PD-L1.
18. The pharmaceutical composition of any one of claims 13-17, further comprising an antibody or antibody fragment against an antigen selected from PD1, PD-L1, AXL, ROR2, CD3, HER2, B7-H3, ROR1, SFRP4 and a WNT protein.
19. The pharmaceutical composition of claim 18, wherein the WNT protein is selected from WNT1, WNT2, WNT2B, WNT3, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9B, WNT10A, WNT10B, WNT11 and WNT16.

20. An anti-CTLA4 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises three complementarity determining regions having amino acid sequences of SEQ ID NOS:39-41 and the light chain variable region comprises three complementarity determining regions having amino acid sequences of SEQ ID NOS:42-44.

21. The antibody of claim 20, wherein the heavy chain variable region has an amino acid sequence of SEQ ID NO:8 and the light chain variable region has an amino acid sequence of SEQ ID NO:7.

22. The antibody of any one of claims 20-21, wherein the antibody has a higher binding affinity to CTLA4 protein at a value of a condition in a tumor microenvironment in comparison with a different value of a same condition that occurs in a non-tumor microenvironment.

23. The antibody of claim 22, wherein the condition is pH and the pH in the tumor microenvironment is in a range of from 5.0 to 6.8 and the pH in the non-tumor microenvironment is in a range of from 7.0 to 7.6.

24. The antibody of any one of claims 20-23, wherein the antibody has a ratio of binding affinity to the CTLA4 protein at a value of a condition in a tumor microenvironment to a binding affinity to the CTLA4 protein at a different value of the same condition in a non-tumor microenvironment of at least about 1.5:1, at least about 2:1, at least about 3:1, at least about 4:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 8:1, at least about 9:1, at least about 10:1, at least about 20:1, at least about 30:1, at least about 50:1, at least about 70:1, or at least about 100:1.

25. The antibody of any one of claims 20-24, wherein the antibody is a chimeric antibody or a humanized antibody.

26. An immunoconjugate comprising the antibody of any one of claims 20-25.

27. The immunoconjugate of claim 26, wherein the immunoconjugate comprises at least one agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.

28. The immunoconjugate of claim 27, wherein the antibody and the at least one agent are covalently bonded to a linker molecule, and the at least one agent is selected from maytansinoids, auristatins, dolastatins, calicheamicin, pyrrolobenzodiazepines, and anthracyclines.

29. A pharmaceutical composition comprising:
the antibody of any one of claims 20-25, or the immunoconjugate of any one of claims 26-28; and
a pharmaceutically acceptable carrier, and optionally, further comprising a tonicity agent.