number of preclinical and clinical studies. Two antibodies against CTLA-4 are in clinical development. Ipilimumab (MDX-010, BMS-734016), a fully human anti-CTLA-4 monoclonal antibody of IgG1-kappa isotype, is an immunomodulatory agent that has been approved as monotherapy for treatment of advanced melanoma. The proposed mechanism of action for Ipilimumab is interference of the interaction of CTLA-4, expressed on a subset of activated T cells, with CD80/CD86 molecules on professional antigen presenting cells. This results in T-cell potentiation due to blockade of the inhibitory modulation of T-cell activation promoted by the CTLA-4 and CD80/CD86 interaction. The resulting T-cell activation, proliferation and lymphocyte infiltration into tumors, leads to tumor cell death. The commercial dosage form is a 5 mg/mL concentrate for solution for infusion. Ipilimumab is also under clinical investigation of other tumor types, including prostate and lung cancers. Another anti-CTLA-4 antibody Tremelimumab was evaluated as monotherapy in melanoma and malignant mesothelioma.

Summary

The present disclosure provides isolated antibodies, in particular monoclonal antibodies or humanized monoclonal antibodies.

In one aspect, the present disclosure refers to an antibody or antigen binding fragment thereof, comprising

a light chain variable region comprising a light chain complementary-determining region 1 (LCDR1) defined by SEQ ID NO: 41, LCDR2 defined by SEQ ID NO: 32, and LCDR3 defined by SEQ ID NO: 22, and

a heavy chain variable region comprising a heavy chain complementary determining region 1 (HCDR1) defined by SEQ ID NO: 36, HCDR2 defined by SEQ ID NO: 28, and HCDR3 defined by SEQ ID NO: 18; and

wherein the antibody or the antigen binding fragment specifically binds to CTLA-4.

In another example, the present disclosure refers to an antibody or an antigen binding-fragment thereof, wherein the antibody or the antigen binding-fragment binds to human, monkey and mouse CTLA-4.

The aforesaid antibody or the antigen binding-fragment inhibits CTLA-4 binding to CD80 or CD86.

In the aforesaid antibody or the antigen binding-fragment, binding

CTLA-4.

The present disclosure provides an antibody or an antigen binding fragment thereof, comprising an amino acid sequence selected from a group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14,

wherein the antibody or the antigen binding-fragment specifically binds to CTLA-4.

In another example, the present disclosure refers to an antibody, or an antigen-binding fragment thereof, comprising:

a) a variable region of a heavy chain having an amino acid sequence that is at least 70%, 80%, 90% or 95% homologous to a sequence selected from a group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, and 7; and

b) a variable region of a light chain having an amino acid sequence that is at least 70%, 80%, 90% or 95% homologous to a sequence selected from a group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13, and 14, wherein the antibody or the antigen binding-fragment specifically binds to CTLA-4.

The present disclosure provides an antibody or an antigen binding fragment thereof, comprising:

a) a variable region of a heavy chain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, and 7; and

b) a variable region of a light chain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13, and 14,

wherein the antibody or the antigen binding-fragment specifically binds to CTLA-4.

The disclosure provides a transgenic animal such as rat comprising human immunoglobulin heavy and light chain transgenes, wherein the rat expresses the antibody of this disclosure.

In another aspect, the present disclosure refers to a transgenic rat comprising human immunoglobulin heavy and light chain transgenes, wherein the rat expresses the antibody as disclosed herein.

The disclosure provides hybridoma prepared from the rat of this disclosure, wherein the hybridoma produces said antibody.

In a further example, the disclosure provides pharmaceutical composition comprising the antibody, or the antigen binding fragment of said antibody in the disclosure, and one or more of a pharmaceutically acceptable excipient, a diluent or a carrier.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising the antibody or the antigen binding fragment thereof as disclosed herein, and one or more of a pharmaceutically acceptable excipient, a diluent and a carrier.

The disclosure provides an immunoconjugate comprising said antibody, or antigen-binding fragment thereof in this disclosure, linked to a therapeutic agent.

In another aspect, the present disclosure refers to an immunoconjugate comprising the antibody or the antigen binding fragment thereof as disclosed herein, linked to a therapeutic agent.

Wherein, the disclosure provides a pharmaceutical composition comprising said immunoconjugate and one or more of a pharmaceutically acceptable excipient, a diluent or a carrier.

In another example, the present disclosure refers to a method for preparing an anti-CTLA-4 antibody or an antigen-binding fragment thereof comprising:

(a) providing:

(i) a heavy chain variable region antibody sequence comprising a CDR1 sequence that is selected from a group consisting of SEQ ID NOs: 33-36, a CDR2 sequence that is selected from a group consisting of SEQ ID NOs: 23-28; and a CDR3 sequence that is selected from the group consisting of SEQ ID NOs: 15-18; and/or

(ii) a light chain variable region antibody sequence comprising a CDR1 sequence that is selected from the group consisting of SEQ ID NOs: 37-41, a

CDR2 sequence that is selected from the group consisting of SEQ ID NOs: 29-32, and a CDR3 sequence that is selected from the group consisting of SEQ ID NOs: 19-22; and

(b) expressing the altered antibody sequence as a protein.

In another aspect, the present disclosure refers to a method for preparing an anti-CTLA-4 antibody or an antigen-binding fragment thereof comprising:

(a) providing:

(i) a heavy chain variable region antibody sequence comprising a CDR1 sequence of SEQ ID NO: 36, a CDR2 sequence of SEQ ID NO: 28; and a CDR3 sequence of SEQ ID NO: 18; and/or

(ii) a light chain variable region antibody sequence comprising a CDR1 sequence of SEQ ID NO: 41, a CDR2 sequence of SEQ ID NO: 32, and a CDR3 sequence of SEQ ID NO: 22; and

(b) expressing the altered antibody sequence as a protein.

In another aspect, the present disclosure refers to the antibody or antigen binding fragment thereof as disclosed herein for use as a medicament.

The disclosure also provides a method of modulating an immune response in a subject comprising administering to the subject the antibody, or antigen binding fragment of any one of said antibodies in this disclosure.

In another example, the present disclosure refers to use of the antibody or the antigen binding fragment thereof as disclosed herein in the manufacture of a medicament for the treatment or prophylaxis of an immune disorder or cancer.

In another aspect, the present disclosure refers to use of the antibody or the antigen binding fragment thereof as disclosed herein in the manufacture of a medicament for the treatment of an immune disorder or cancer.

The disclosure also provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of said antibody, or said antigen-binding fragment to inhibit growth of the tumor cells.

In another aspect, the present disclosure refers to use of the antibody or the antigen binding fragment thereof as disclosed herein in the manufacture of a medicament for inhibiting growth of tumor cells.

Wherein, the disclosure provides the method, wherein the tumor cells are of a cancer selected from a group consisting of melanoma, renal cancer, prostate cancer, breast cancer, colon cancer, lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, and rectal cancer.

Wherein, the disclosure provides the method, wherein the antibody is a chimeric antibody, humanized antibody, human antibody or rat antibody.

The features and advantages of this disclosure

The inventors have generated humanized antibodies against CTLA-4

Claims

1. An antibody or antigen binding fragment thereof, comprising

   a light chain variable region comprising a light chain complementary-determining region 1 (LCDR1) defined by SEQ ID NO: 41, LCDR2 defined by SEQ ID NO: 32, and LCDR3 defined by SEQ ID NO: 22, and

   a heavy chain variable region comprising a heavy chain complementary determining region 1 (HCDR1) defined by SEQ ID NO: 36, HCDR2 defined by SEQ ID NO: 28, and HCDR3 defined by SEQ ID NO: 18; and

   wherein the antibody or the antigen binding fragment specifically binds to CTLA-4.

2. The antibody or an antigen binding fragment thereof according to claim 1, comprising an amino acid sequence that is at least 90% or 95% homologous to SEQ ID NO: 7 or 14.

3. The antibody or antigen binding fragment thereof according to claim 1 or 2, wherein the light chain variable region has the amino acid sequence of SEQ ID NO: 14.

4. The antibody or antigen binding fragment thereof according to any one of claims 1-3, wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO: 7.

5. The antibody or antigen binding fragment thereof according to claim 4, wherein the light chain variable region has the amino acid sequence of SEQ ID NO: 14 and the heavy chain variable region has the amino acid sequence of SEQ ID NO: 7.

6. The antibody or the antigen binding-fragment thereof according to any one of claims 1-5, wherein the antibody inhibits CTLA-4 binding to CD80 or CD86.

7. The antibody or the antigen binding-fragment thereof according to any one of claims 1-6, wherein binding epitope of antibody or antigen binding-fragment comprises N145, polysaccharide on N145, or P138 of CTLA-4.

8. The antibody or an antigen binding fragment thereof according to any one of claims 1-7, wherein the antibody or the antigen binding-fragment
   a) binds to human CTLA-4 with a K_D of 4.77E-10 M or less; and
   b) binds to mouse CTLA-4 with a K_D of l.39E-09 M or less.

9. The antibody or the antigen binding fragment thereof according to any one of claims 1-8, wherein the antibody is chimeric, humanized, fully human, or rat antibody.

10. A nucleic acid molecule encoding the antibody or the antigen binding fragment thereof according to any one of claims 1 to 9.

11. A cloning or expression vector comprising the nucleic acid molecule of claim 10.

12. A host cell comprising one or more expression vectors of claim 11.

13. A process for the production of the antibody of any one of claims 1-9, comprising culturing the host cell of claim 12 and isolating the antibody.

14. A transgenic rat comprising human immunoglobulin heavy and light chain transgenes, wherein the rat expresses the antibody of any one of claims 1-9.

15. A pharmaceutical composition comprising the antibody or the antigen binding fragment thereof according to any one of claims 1 to 9, and one or more of a pharmaceutically acceptable excipient, a diluent and a carrier.

16. An immunoconjugate comprising the antibody or the antigen binding fragment thereof according to any one of claims 1-9, linked to a therapeutic agent.

17. A method for preparing an anti-CTLA-4 antibody or an antigen-binding fragment thereof comprising:

(a) providing:

(i) a heavy chain variable region antibody sequence comprising a CDR1 sequence of SEQ ID NO: 36, a CDR2 sequence of SEQ ID NO: 28; and a CDR3 sequence of SEQ ID NO: 18; and/or

(ii) a light chain variable region antibody sequence comprising a CDRl sequence of SEQ ID NO: 41, a CDR2 sequence of SEQ ID NO: 32, and a CDR3 sequence of SEQ ID NO: 22; and

(b) expressing the altered antibody sequence as a protein.

18. The antibody or antigen binding fragment thereof according to any one of claims 1 to 9 for use as a medicament.

19. Use of the antibody or the antigen binding fragment thereof according to any one of claims 1 to 9 in the manufacture of a medicament for the treatment of an immune disorder or cancer.

20. Use of the antibody or the antigen binding fragment thereof according to any one of claims 1 to 9 in the manufacture of a medicament for inhibiting growth of tumor cells.

21. The use of claim 20, wherein the tumor cells are of a cancer selected from a group consisting of melanoma, renal cancer, prostate cancer, breast cancer, colon cancer, lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, and rectal cancer.