IL-2 from human lymphocytes. The antibodies 14C12 and 14C12H1L1 herein have the potential to become drugs for preventing and treating malignancies including lung cancer, melanoma, renal cancer, ovarian cancer and leukemia, as well as anemia.

The following are provided by the present disclosure:

In one aspect, the present disclosure refers to a monoclonal antibody or antigen-binding fragment thereof, wherein

the heavy chain variable region (V_H) of the said monoclonal antibody comprises CDRs with the amino acid sequences of SEQ ID NO:9-11;

and

the light chain variable region (V_L) of the said monoclonal antibody comprises CDRs with the amino acid sequences of SEQ ID NO:12-14.

In some examples of the present disclosure, the said monoclonal antibody or its antigen-binding fragments thereof, wherein,

the amino acid sequence of V_H of the monoclonal antibody is chosen from SEQ ID NO:2 and SEQ ID NO:6;

and/or

the amino acid sequence of V_L of the monoclonal antibody is chosen from SEQ ID NO:4 and SEQ ID NO:8.

In an example of the present disclosure, the said monoclonal antibody or its antigen-binding fragments thereof, wherein, the monoclonal antibody comprises:

(1) V_H shown by SEQ ID NO:2 and V_L shown by SEQ ID NO:4;

(2) V_H shown by SEQ ID NO:6 and VL shown by SEQ ID NO:8.

The variable regions in heavy and light chains of an antibody govern binding activity. Each chain contains three hypervariable regions, namely, the complementary determining region (CDR) (HCDR1, HCDR2 and HCDR3 in heavy (H) chain, and LCDR1, LCDR2 and

LCDR3 in light (L) chain), which are defined by Kabat, et al. (Sequences of Proteins of Immunological Interest, Fifth Edition (1991), volume 1-3, NIH Publication 91-3242, Bethesda MD).

Through techniques well-known to technical personnel in the field described herein, for example, analyzing the amino acid sequences in the CDR of the monoclonal antibody sequences in Items (1) and (2) above through VBASE2 database:

The antibodies 14C12 and 14C12H1L1 herein comprises the same CDR:

Wherein the amino acid sequences of the 3 CDR regions of V_H are as follows:

HCDR1: GFAFSSYD (SEQ ID NO:9),

HCDR2: ISGGGRYT (SEQ ID NO:10),

HCDR3: ANRYGEAWFAY (SEQ ID NO:11);

Wherein the amino acid sequences of the 3 CDR regions of V_L are as follows:

LCDR1: QDINTY (SEQ ID NO:12),

LCDR2: RAN (SEQ ID NO:13),

LCDR3: LQYDEFPLT (SEQ ID NO:14).

In certain example, the said monoclonal antibody or its antigen-binding fragment thereof, wherein the said monoclonal antibody or its antigen-binding fragment are selected from Fab, Fab', F(ab')2, Fd, Fv, dAb, CDRs, single chain antibodies (e.g. scFv), humanized antibodies, chimeric antibodies, or bispecific antibodies.

In certain examples, the said monoclonal antibody or its antigen-binding fragment thereof, wherein the said monoclonal antibody binds to PD1 protein with K_D less than approximately 10^{-5} M, for example, less than approximately 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, or less; preferably, detected by Fortebio molecular interaction equipment.

In certain examples, the said monoclonal antibody or its antigen-binding fragment thereof, wherein the said monoclonal antibody binds to PD1 protein with EC_{50} less than

approximately 100 nM, for example, less than 10 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM or less. Specifically, the said EC_{50} is determined by an indirect ELISA.

In certain examples, the said monoclonal antibody or its antigen-binding fragment thereof, wherein the said monoclonal antibody binds to PD1 protein with K_D less than approximately 10^{-5} M, such as less than approximately 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, or less,

In certain examples, the said monoclonal antibody or its antigen-binding fragment thereof, wherein the said monoclonal antibody contains non-CDR regions from species other than mouse, for example, from human.

In certain examples, the said monoclonal antibody or its antigen-binding fragments thereof, wherein the said monoclonal antibody is produced by the hybridoma cell line LT003, and the said hybridoma cell line LT003 is preserved in China Center for Type Culture Collection (CCTCC) with the CCTCC Deposit Accession NO: C2015105.

Disclosed is an isolated nucleic acid molecule comprising a nucleotide sequence capable of encoding V_H of the antibody, wherein,

the V_H of the said antibody comprises: CDRs with the amino acid sequences from SEQ ID NO:9-11;

Specifically, the heavy chain of the said antibody has the amino acid sequences from SEQ ID NO:2 and SEQ ID NO:6;

More specifically, the said nucleic acid molecule has the nucleotide sequences from SEQ ID NO:1 or SEQ ID NO:5.

In another aspect, the present disclosure refers to an isolated nucleic acid molecule comprising a nucleotide sequence coding for a heavy chain variable region (V_H) of a monoclonal antibody comprising CDRs with the amino acid sequences SEQ ID NO: 9-11; preferably, a nucleotide sequence coding for a V_H of a monoclonal antibody comprising amino acid sequence SEQ ID NO: 2 or SEQ ID NO: 6; preferably, wherein the nucleic acid molecule comprises the nucleotide sequence SEQ ID NO: 1 or SEQ ID NO: 5.

Disclosed is an isolated nucleic acid molecule comprising a nucleotide sequence capable of encoding V_L of the antibody, wherein,

the V_L of the antibody comprises CDRs with the amino acid sequences from SEQ ID NO:12-14;

Specifically, the V_L of the said antibody has the amino acid sequences from SEQ ID NO:4 or SEQ ID NO:8;

More specifically, the said nucleic acid molecule has the nucleotide sequences from SEQ ID NO:3 or SEQ ID NO:7.

In another example, there is provided an isolated nucleotide, containing a sequence coding for a light chain variable fragment with CDRs having amino acid sequences SEQ ID NO: 12-14; Preferably, the light chain variable fragment comprises an amino acid sequence chosen from SEQ ID NO: 4 and SEQ ID NO: 8; and more preferably, the light chain variable fragment is encoded by a nucleotide sequence SEQ ID NO: 3 or SEQ ID NO: 7.

Disclosed is a vector comprising the isolated nucleic acid molecule described in the present disclosure.

In another aspect, the present disclosure refers to an expression construct comprising the isolated nucleic acid molecule as described herein; optionally further comprising an isolated nucleic acid molecule comprising a nucleotide sequence coding for a light chain variable region (V_L) of a monoclonal antibody comprising CDRs with the amino acid sequences from SEQ ID NO: 12-14; preferably, a nucleotide sequence coding for a V_L of a monoclonal antibody comprising amino acid sequence SEQ ID NO: 4 or SEQ ID NO: 8; preferably, wherein the nucleic acid molecule comprises the nucleotide sequence SEQ ID NO: 3 or SEQ ID NO: 7.

Disclosed is a host cell comprising the isolated nucleic acid molecule described in the present disclosure, or vector described in the present disclosure.

In another aspect, the present disclosure refers to an expression cell line comprising the isolated nucleic acid molecule as described herein, or the expression construct as described herein.

Disclosed is a method for preparing the monoclonal antibody or its antigen-binding fragments thereof described in the present disclosure, by culturing the host cell in the present disclosure under appropriate conditions, and recovering the said monoclonal antibody or its antigen-binding fragments thereof from the cell culture.

In another aspect, the present disclosure refers to a method of producing the monoclonal antibody or antigen binding fragment as described herein, the method comprising growing the cell line as described herein under suitable conditions, and recovering the monoclonal antibody or antigen binding fragment from the cell culture.

Disclosed is the hybridoma cell line LT003 that is preserved in the China Center for Typical Culture Collection (CCTCC) with the CCTCC Deposit Accession NO: C2015105.

In another aspect, the present disclosure refers to hybridoma cell line LT003, preserved in the China Center for Typical Culture Collection (CCTCC) with the CCTCC Deposit Accession NO: C2015105.

Disclosed is a conjugate that consist of monoclonal antibody or its antigen binding fragments and conjugating part, wherein, the said monoclonal antibody is monoclonal antibody or its antigen binding fragments described in the present disclosure, and the said conjugating part as a detectable marker. Specifically, the said conjugating part are radioactive isotopes, fluorescein, luminescent materials, colorful substances, or enzymes.

In another aspect, the present disclosure refers to a conjugate comprising the monoclonal antibody or antigen binding fragment as described herein and a conjugating partner as a detectable marker; preferably wherein the conjugating partner comprises radioactive isotopes, fluorescein, luminescent material, colorful substances, or enzymes.

Disclosed are reagent kits, consisting of the monoclonal antibody or its antigen binding fragments, or the conjugates thereof described in the disclosure;

In another aspect, the present disclosure refers to a reagent kit comprising the monoclonal antibody or antigen binding fragment as described herein, or the conjugate as described herein; preferably wherein the kit comprises a secondary antibody, which specifically recognizes the monoclonal antibody or antigen binding fragment; optionally wherein the secondary antibody comprises a detectable marker, preferably wherein the detectable marker comprises radioactive isotopes, fluorescein, luminescent material, colorful substances, or enzymes.

Specifically, the reagent kits may contain a secondary antibody, which specifically recognizes the said monoclonal antibody or its antigen binding fragments; optionally, the said secondary antibody may contain detectable markers, such as radioactive isotopes, fluorescein, luminescent materials, colorful substances, or enzymes.

Disclosed is use of the said monoclonal antibody or its antigen binding fragments, or the conjugates thereof described in the disclosure in preparation of reagent kits, the said reagent kits are used in detection of the existence or the level of PD1 in samples.

In another aspect, the present disclosure refers to use of the monoclonal antibody, or its antigen binding fragments thereof, as described herein, or the conjugate as described herein, for the manufacturing of a kit for the detection of the existence or the levels of PD-1 in a sample.

Disclosed is a pharmaceutical composition comprising the said monoclonal antibody or its antigen binding fragments, or the conjugates thereof described in the disclosure. Optionally, it may also comprise a pharmaceutically acceptable carrier or excipient.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising the monoclonal antibody or antigen binding fragment as described herein or a conjugate as described herein; optionally wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier or excipient.

Disclosed is use of the said monoclonal antibody or its antigen binding fragments or the conjugates thereof described in the disclosure in preparing drugs for prevention and/or treatment and/or adjuvant treatment and/or diagnosis of tumors or anemia; specifically, the said tumors may be melanoma, renal cancer, prostate cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, liver cancer, non-small cell lung cancer, ovarian cancer and leukemia.

In another aspect, the present disclosure refers to use of the monoclonal antibody or antigen binding fragment as described herein, or the conjugate as described herein, or the pharmaceutical composition as described herein, for the manufacturing of a medicament for prevention and/or treatment of a tumor or anemia in a subject in need thereof; preferably wherein the tumor may be melanoma, renal cancer, prostate cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, liver cancer, non-small cell lung cancer, ovarian cancer or leukemia.

The present inventors have found through animal experiments that, 14C12H1L1 can effectively inhibit the growth of MC38 tumor cells inoculated at right side subcutaneously in PD-1 HuGEMM mice, which the antibody drug 14C12H1L1 can significantly inhibit the tumor growth in PD-1 HuGEMM tumor-bearing mice, having an efficacy equivalent to the marketed monoclonal antibody drug Nivolumab that is approved drug targeting the same target.

Disclosed is use of the monoclonal antibody or its antigen-binding fragments or the conjugates thereof described in the present disclosure for preparation drugs

with the following purposes:

Blocking the binding of PD1 to PD1 ligand,

Regulating (e.g. Down-regulating) PD1 activity or level,

Relieving the immunosuppression of PD1, or

Up-regulating IFN-γ and/or IL-2 expressions in T lymphocytes;

Specifically, the said PD1 ligand is PDL1 or PDL2, preferably PDL1.

In another example, there is provided use of the monoclonal antibody or its antigen binding fragments as described herein, or the conjugate as described herein, for the manufacturing of a medicament selected from the following:

a medicament for blocking the binding of PD1 to PD1 ligand,

a medicament for regulating (e.g. Down-regulating) PD1 activity or level,

a medicament for relieving the immunosuppression of PD1, or

a medicament for up-regulating IFN-γ and/or IL-2 expressions in T lymphocytes;

Preferably, the PD1 ligand is PDL1 or PDL2, more preferably PDL1.

Disclosed is an *in vivo* or *in vitro* method to apply to cells or subjects in need with an effective dose of the monoclonal antibody or its antigen-binding fragments or the conjugates thereof described in the present disclosure, and the said method is selected from the following:

Methods to block the binding of PD1 to PD1 ligand,

Methods to regulate (e.g. down-regulate) PD1 activity or level,

Methods to relieve the immunosuppression of PD1, or

Methods to up-regulate IFN-γ and/or IL-2 expressions in T lymphocytes;

Specifically, the said PD1 ligand is PDL1 or PDL2, preferably PDL1.

In another example, there is provided an in vivo or in vitro method, comprising administering to a cell or subjects in need with an effective dose of the monoclonal antibody or its antigen binding fragments as described herein, or the conjugate as described herein, for the

In a specific example of the present disclosure, the monoclonal antibody or its antigen-binding fragments or the conjugates thereof described in the present disclosure only blocks the binding of PD1 to PDL1.

Disclosed is a method for the prevention and/or treatment and/or adjuvant treatment and/or diagnosis of tumors or anemia, including the procedure to apply to subjects with an effective dose of the monoclonal antibody or its antigen-binding fragments or the conjugates thereof described in the present disclosure; specifically, the said tumors may be melanoma, renal cancer, prostate cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, liver cancer, non-small cell lung cancer, ovarian cancer or leukemia.

In another example, there is provided use of the monoclonal antibody or its antigen binding fragments as described herein, or the conjugate as described herein, for the manufacturing of a medicament for prevention and/or treatment of tumors or anemia, or diagnosis of tumors or anemia in a subject in need thereof; preferably, the tumors may be melanoma, renal cancer, prostate cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, liver cancer, non-small cell lung cancer, ovarian cancer and leukemia.

In another aspect, the present disclosure refers to the monoclonal antibody or antigen binding fragment as described herein, or the conjugate as described herein, or the pharmaceutical composition as described herein, for use in therapy.

In another aspect, the present disclosure refers to the monoclonal antibody or antigen binding fragment as disclosed herein, or the conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, for use in the prevention and/or treatment of a tumor or anemia, preferably wherein the tumor is melanoma, renal cancer, prostate cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, liver cancer, non-small cell lung cancer, ovarian cancer or leukemia.

Unless otherwise defined herein, scientific and technical terms used in connection with the

CLAIMS

1. A monoclonal antibody or antigen-binding fragment thereof, wherein

the heavy chain variable region (V_H) of the monoclonal antibody comprises CDRs with the amino acid sequences of SEQ ID NO:9-11;

and

the light chain variable region (V_L) of the monoclonal antibody comprises CDRs with the amino acid sequences of SEQ ID NO:12-14.

2. The monoclonal antibody or antigen-binding fragment of claim 1, wherein

the amino acid sequence of the V_H of the monoclonal antibody is chosen from SEQ ID NO:2 and SEQ ID NO:6;

and

the amino acid sequence of the V_L of the monoclonal antibody is chosen from SEQ ID NO:4 and SEQ ID NO:8;

optionally wherein the monoclonal antibody comprises a V_H amino acid sequence of SEQ ID NO:2 and a V_L amino acid sequence of SEQ ID NO:4; or a V_H amino acid sequence of SEQ ID NO:6 and a V_L amino acid sequence of SEQ ID NO:8.

3. The monoclonal antibody, or antigen-binding fragment of claim 1 or 2, wherein the monoclonal antibody or antigen-binding fragment is a Fab, Fab', $F(ab')_2$, Fd, Fv, dAb, single-chain antibody, scFv, humanized antibody, chimeric antibody, or bispecific antibody.

4. The monoclonal antibody or antigen-binding fragment of claim 1 or 2, wherein the binding affinity (K_D) to PD1 of the monoclonal antibody or antigen-binding fragment is less than 10^{-5} M, preferably 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, or 10^{-10} M or less; preferably wherein the K_D is

measured by Fortebio molecular interactions analyzer.

5. The monoclonal antibody or antigen-binding fragment of claim 1 or 2, wherein the monoclonal antibody or antigen-binding fragment contains non-CDR regions from species other than mouse, preferably human.

6. The monoclonal antibody or antigen-binding fragment of claim 1 or 2, wherein the monoclonal antibody or antigen-binding fragment is produced by the hybridoma cell line LT003 preserved in China Center for Type Culture Collection (CCTCC) with the CCTCC Deposit Accession NO: C2015105.

7. An isolated nucleic acid molecule comprising a nucleotide sequence coding for a heavy chain variable region (V_H) of a monoclonal antibody comprising CDRs with the amino acid sequences SEQ ID NO: 9-11; preferably, a nucleotide sequence coding for a V_H of a monoclonal antibody comprising amino acid sequence SEQ ID NO: 2 or SEQ ID NO: 6; preferably, wherein the nucleic acid molecule comprises the nucleotide sequence SEQ ID NO: 1 or SEQ ID NO: 5.

8. An expression construct comprising the isolated nucleic acid molecule of claim 7;

optionally further comprising an isolated nucleic acid molecule comprising a nucleotide sequence coding for a light chain variable region (V_L) of a monoclonal antibody comprising CDRs with the amino acid sequences from SEQ ID NO: 12-14; preferably, a nucleotide sequence coding for a V_L of a monoclonal antibody comprising amino acid sequence SEQ ID NO: 4 or SEQ ID NO: 8; preferably, wherein the nucleic acid molecule comprises the nucleotide sequence SEQ ID NO: 3 or SEQ ID NO: 7.

9. An expression cell line comprising the isolated nucleic acid molecule of claim 7 or the expression construct of claim 8.

10. A method of producing the monoclonal antibody or antigen binding fragment of any one of claims 1 to 6, the method comprising growing the cell line of claim 9 under suitable conditions, and recovering the monoclonal antibody or antigen binding fragment from the cell culture.

11. Hybridoma cell line LT003, preserved in the China Center for Typical Culture Collection (CCTCC) with the CCTCC Deposit Accession NO: C2015105.

12. A conjugate comprising the monoclonal antibody or antigen binding fragment of any one of claims 1 to 6 and a conjugating partner as a detectable marker; preferably wherein the conjugating partner comprises radioactive isotopes, fluorescein, luminescent material, colorful substances, or enzymes.

13. A reagent kit comprising the monoclonal antibody or antigen binding fragment of any one of claims 1 to 6, or the conjugate of claim 12; preferably wherein the kit comprises a secondary antibody, which specifically recognizes the monoclonal antibody or antigen binding fragment; optionally wherein the secondary antibody comprises a detectable marker, preferably wherein the detectable marker comprises radioactive isotopes, fluorescein, luminescent material, colorful substances, or enzymes.

14. Use of the monoclonal antibody, or its antigen binding fragments thereof, of any one of claims 1 to 6, or the conjugate of claim 12, for the manufacturing of a kit for the detection of the existence or the levels of PD-1 in a sample.

15. A pharmaceutical composition comprising the monoclonal antibody or antigen binding fragment of any one of claims 1 to 6 or a conjugate of claim 12; optionally wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier or excipient.

16. Use of the monoclonal antibody or antigen binding fragment of any one of claims 1 to 6, or the conjugate of claim 12, or the pharmaceutical composition of claim 15, for the manufacturing of a medicament for prevention and/or treatment of a tumor or anemia in a subject in need thereof; preferably wherein the tumor may be melanoma, renal cancer, prostate cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, liver cancer, non-small cell lung cancer, ovarian cancer or leukemia.

17. The monoclonal antibody or antigen binding fragment of any one of claims 1 to 6, or the conjugate of claim 12, or the pharmaceutical composition of claim 15, for use in therapy.

18. The use of claim 16, wherein an effective dose of the monoclonal antibody or its antigen-binding fragments of any one of claims 1 to 6, or the conjugate of claim 13 is to be administered to the subject; preferably, the tumors may be melanoma, renal cancer, prostate cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, liver cancer, non-small cell lung cancer, ovarian cancer or leukemia.

19. The monoclonal antibody or antigen binding fragment of any one of claims 1 to 6, or the conjugate of claim 12, or the pharmaceutical composition of claim 15, for use in the prevention and/or treatment of a tumor or anemia, preferably wherein the tumor is melanoma, renal cancer, prostate cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, liver cancer, non-small cell lung cancer, ovarian cancer or leukemia.