generally believed that antibodies against PD1 pathways will lead to breakthroughs in the treatment of a variety of tumors: non-small cell lung cancer, renal cell carcinoma, ovarian cancer, melanoma (Homet M.B., Parisi G., et al., Anti-PD1 Therapy in Melanoma. Semin Oncol. 2015 Jun;42(3):466-473), leukemia, and anemia (Held SA, Heine A, et al., Advances in immunotherapy of chronic myeloid leukemia CML. Curr Cancer Drug Targets. 2013 Sep;13(7):768-74).

Ever since the revelation of the unprecedented clinical efficacy data at the annual meetings of American Association for Cancer Research (AACR) and American Society of Clinical Oncology (ASCO) in 2012 and 2013, PD1 antibodies have become the hottest new drugs in R&D in the global pharmaceutical industry.

At present, there is still a need to develop new anti-PD1 antibodies with better binding efficiency to effectively block the binding of PD1 to PDL1.

Summary

Through in-depth research and creative work, by immunizing mice with recombinant PD1 expressed in mammalian cells expression system as antigen, the inventors obtained hybridoma cells via fusion of mouse splenocytes and myeloma cells. By screening a large number of samples, the inventors obtained the hybridoma cell line LT003 (CCTCC Deposit Accession No.: C2015105).

The inventors surprisingly found that the hybridoma cell line LT003 is capable of secreting a specific monoclonal antibody (named 14C12) binding specifically to PD1, and this monoclonal antibody can effectively block the association of PD1 to PDL1.

Furthermore, the inventors generated a humanized anti-PD1 antibody (named 14C12H1L1) in a creative way.

More surprisingly, the inventors found that the antibodies 14C12 and 14C12H1L1 herein can effectively bind to human T cells, activate T cells and induce the secretion of IFN-γ and

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, there is provided a monoclonal antibody or its antigen-binding fragments 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/or

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

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 embodiments, 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 embodiments, 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 embodiments, 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, there is provided an isolated nucleotide, containing a sequence coding for a heavy chain variable fragment with CDRs having amino acid sequences SEQ ID NO: 9-11; Preferably, the heavy chain variable fragment comprises an amino acid sequence chosen from SEQ ID NO: 2 and SEQ ID NO: 6; and more preferably, the heavy chain variable fragment is encoded by a nucleotide sequence of 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 aspect, 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, there is provided an expression construct, comprising the expression construct comprises the isolated nucleotide as described herein.

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, there is provided an expression cell line, comprising the isolated nucleotide 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, there is provided a method of producing the monoclonal antibody or its antigen binding fragments as described herein, comprising growing the cell line as described herein under suitable conditions, and recovering monoclonal antibodies or its antigen binding fragments 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, there is provided hybridoma cell line LT003, wherein the hybridoma cell line has been deposited 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, there is provided a conjugate, comprising the monoclonal antibody or its antigen binding fragments as described herein, and a conjugating partner as a detectable marker; Preferably, the conjugating partner is selected from the group consisting of radioactive isotopes, fluorescein, luminescent material, colorful substances, and 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, there is provided a reagent kit, comprising the monoclonal antibody or its antigen binding fragments as described herein, or the conjugate as described herein; Preferably, the kit may contain a secondary antibody, wherein the secondary antibody specifically recognizes the monoclonal antibody or its antigen binding fragments; Optionally, the secondary antibody may contain a detectable marker.

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, 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 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, there is provided a pharmaceutical composition, comprising the monoclonal antibody or its antigen binding fragments as described herein, or the conjugate as described herein; optionally, 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, 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 or treatment of a tumor or anemia, or for diagnosing a tumor or anemia; preferably, 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 aspect, 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 aspect, 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

purpose selected from the following:

blocking the binding of PD1 to PD1 ligand,

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

relieving the immunosuppression of PD1, or

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

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

In a specific example of the present disclosure, the said *in vitro* method is intended for non-therapeutic or -diagnostic purposes.

Interferon γ (IFNγ), is mainly naturally produced by natural killer (NK) cells and natural killer T (NKT) cells, or produced by effector T cells consisting of CD4 Th1 cells and CD8 cytotoxic T lymphocytes after being stimulated by specific antigens. As an important cytokine of innate and acquired immune, IFNγ plays an import role in antagonizing or inhibiting viral, some bacterial and protozoon infections. In the meantime, IFNγ can activate macrophages and induce the expression of type 2 major histocompatibility complex (MHC) to activate immune responses to control the progression of tumors (Schoenborn JR, Wilson CB. Regulation of

Interferon-γ During Innate and Adaptive Immune Responses. Advances in Immunology 2007; 96:41-101). In the in vitro study of the present disclosure, the anti-PD1 antibody can induce the secretion of IFNγ to activate immune responses.

Interleukin 2 (IL-2) produced by T cells is a growth factor regulating T cell subsets and a crucial factor regulating immune responses, promoting activated B cells proliferation, and participating in antibody responses, hematopoiesis and oncological surveillance. Recombinant human IL-2 has been approved by the U. S. FDA for the treatment of malignant tumors (including melanoma, renal tumor, etc.) while undergoing clinical studies for the treatment of chronic viral infections (Chavez, A.R., et al., Pharmacologic administration of interleukin-2. Ann N Y Acad Sci, 2009.1182:p.14-27). In vitro studies, the anti-PD1 antibody of the present disclosure can specifically relieve the immunosuppression of PD1, activate T cells and induce IL-2 production, displaying promising prospects of extensive applications in gene therapies for neoplastic and parasitic diseases.

The monoclonal antibody or its antigen-binding fragments or the conjugates thereof described in the present disclosure is used for the 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 or leukemia.

The monoclonal antibody or its antigen-binding fragments or the conjugates thereof described in the present disclosure is used to:

Block the binding of PD1 to PD1 ligand,

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

Relieve the immunosuppression of PD1, or

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

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

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 aspect, 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, there is provided the monoclonal antibody or its antigen binding fragments as described herein, or the conjugate as described herein, for use in any one of the following:

Blocking the binding of PD1 to PD1 ligand,

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

Relieving the immunosuppression of PD1, or

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

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

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

present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Furthermore, laboratory techniques of cell and tissue culture, molecular genetics, oligo- or polynucleotide chemistry, and immunology described herein are those well-known and commonly used in the art. Meanwhile, to better understand the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

As used in this invention, the term "Amino acid sequence of PD1 protein (Programmed cell death protein 1, NCBI GenBank: NP_005009.2)" comprises the full length PD1 protein, or PD1ECD (extracellular segment of PD1) or PD1ECD-containing fragments; and also comprises the fusion protein of PD1ECD, for example, fused with the Fc protein fragments of mouse or human IgG (mFc or hFc). Furthermore, understood by those of ordinary skill in the art, the amino acid sequence of PD1 protein can have naturally or artificial mutations (including but not limited to substitutions, deletions, and/or additions), not affecting its biological functions. Therefore, in the present invention, the term "PD1 protein" should include all such sequences and their natural or artificial variants. Furthermore, when describing the sequence fragments of PD1 protein, the said sequence fragments comprise both the sequence fragments and the corresponding sequence fragments in its natural or artificial variants.

As used in this invention, the term "Amino acid sequence of PDL1 protein (Programmed death-ligand 1, NCBI Genebank ID: NP_054862.1)" comprises the full length PDL1 protein, or PDL1ECD (extracellular segment of PDL1) or PDL1ECD-containing fragments; and also comprises the fusion protein of PDL1ECD, for example, fused with the Fc protein fragments of mouse or human IgG (mFc or hFc). Furthermore, understood by those of ordinary skill in the art, the amino acid sequence of PDL1 protein can have naturally or artificial mutations (including but not limited to substitutions, deletions, and/or additions), not affecting its biological functions. Therefore, in the present invention, the term "PDL1 protein" should include

CLAIMS

1. A monoclonal antibody or its antigen-binding fragments 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/or

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. A monoclonal antibody or its antigen-binding fragments thereof described in Claim 1, wherein,

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

and/or

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

3. The monoclonal antibody or its antigen-binding fragments of any one of claims 1 to 2, wherein the sequences or part of the sequences are contained in Fab, Fab', $F(ab')_2$, Fd, Fv, dAb, CDR fragments, single-chain antibodies (e.g. scFv), humanized antibodies, chimeric antibodies, or bispecific antibodies.

4. The monoclonal antibody or its antigen binding fragments of any one of claims 1 to 2, the binding affinity (K_D) to PD1 of which is less than 10^{-5} M, for example, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, or 10^{-10} M or less; preferably, the K_D is measured by Fortebio molecular interactions analyzer.

5. Any of the monoclonal antibodies or its antigen binding fragments from claims 1 to 2, which contains non-CDR regions from species other than mouse, such as human.

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

7. An isolated nucleotide, containing a sequence coding for a heavy chain variable fragment with CDRs having amino acid sequences SEQ ID NO: 9-11; Preferably, the heavy chain variable fragment comprises an amino acid sequence chosen from SEQ ID NO: 2 and SEQ ID NO: 6; and more preferably, the heavy chain variable fragment is encoded by a nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 5.

8. 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.

9. An expression construct, comprising the expression construct comprises the isolated nucleotide of claims 7 and/or 8.

10. An expression cell line, comprising the isolated nucleotide of claims 7 and/or 8, or the

expression construct of claim 9.

11. A method of producing the monoclonal antibody or its antigen binding fragments from claims 1 to 6, comprising growing the cell line from claim 10 under suitable conditions, and recovering monoclonal antibodies or its antigen binding fragments from the cell culture.

12. Hybridoma cell line LT003, wherein the hybridoma cell line has been deposited in the China Center for Typical Culture Collection (CCTCC) with the CCTCC Deposit Accession NO: C2015105.

13. A conjugate, comprising the monoclonal antibody or its antigen binding fragments of any one of claims 1 to 6, and a conjugating partner as a detectable marker; Preferably, the conjugating partner is selected from the group consisting of radioactive isotopes, fluorescein, luminescent material, colorful substances, and enzymes.

14. A reagent kit, comprising the monoclonal antibody or its antigen binding fragments of any one of claims 1 to 6, or the conjugate of claim 13; Preferably, the kit may contain a secondary antibody, wherein the secondary antibody specifically recognizes the monoclonal antibody or its antigen binding fragments; Optionally, the secondary antibody may contain a detectable marker.

15. Use of the monoclonal antibody or its antigen binding fragments of any one of claims 1 to 6 or the conjugate of claim 13, for the manufacturing of a kit for the detection of the existence or the levels of PD-1 in a sample.

16. A pharmaceutical composition, comprising the monoclonal antibody or its antigen binding

fragments of any one of claims 1 to 6, or the conjugate of claim 13; optionally, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier or excipient.

17. Use of the monoclonal antibody or its antigen binding fragments of any one of claims 1 to 6, or the conjugate of claim 13, for the manufacturing of a medicament for prevention or treatment of a tumor or anemia, or for diagnosing a tumor or anemia; preferably, 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.

18. Use of the monoclonal antibody or its antigen binding fragments of any one of claims 1 to 6, or the conjugate of claim 13, 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.

19. 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 of any one of claims 1 to 6, or the conjugate of claim 13, for the purpose selected from the following:

blocking the binding of PD1 to PD1 ligand,

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

relieving the immunosuppression of PD1, or

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

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

20. Use of the monoclonal antibody or its antigen binding fragments of any one of claims 1 to 6, or the conjugate of claim 13, 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.

21. The monoclonal antibody or its antigen binding fragments of any one of claims 1 to 6, or the conjugate of claim 13, for use in any one of the following:

Blocking the binding of PD1 to PD1 ligand,

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

Relieving the immunosuppression of PD1, or

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

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

22. The use of claim 20, 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.