antigens at the same time, can be produced by immune sorting purification, as well as advantageous recombinant technologies with flexibilities in binding site optimization, format of synthesis, and production output. At present, there have been more than 45 forms of bispecific antibodies (Müller D, Kontermann RE. Bispecific antibodies for cancer immunotherapy: Current perspectives. BioDrugs 2010; 24:89-98). The IgG-scFv structure, named after Morrison, has been used in many bispecific antibodies, (1997 Coloma MJ, Morrison SL. The Design and production of will be tetravalent bispecific antibodies. Nat Biotechnol. Nature Biotechnology, 1997; 15, 15, 9-163). Bispecific antibody with IgG-scFv structure has been proven as an ideal form of bispecific antibody with advantages in antibody engineering, expression and purification due to its similarity to the natural IgG format (Miller BR, Demarest SJ, et al., Stability engineering of scFvs for the development of bispecific and multivalent antibodies. Protein Eng Des Sel 2010; 23:549-57; Fitzgerald J, Lugovskoy A. Rational engineering of antibody therapeutics targeting multiple oncogene pathways. MAbs 2011; 3:299-309).

However, bispecific antibodies have been developed mostly against antigens on the surface of two different cells, not against two different antigens on the same cell. Thus, a bispecific antibody drug against both PD-1 and CTLA4 needs to be developed.

SUMMARY

Through profound research and creative work, by immunizing mice with recombinant PD-1 or CTLA4 expressed in mammalian cells as antigen, the inventors obtained hybridoma cells via fusion of mouse splenocytes and myeloma cells. After screening a large number of samples, the inventors obtained the following hybridoma cell lines respectively:

Hybridoma cell line LT002 (CTLA4-4G10), which was preserved in China Center for Type Culture Collection (CCTCC) on June 16, 2015, with the CCTCC Deposit Accession NO: C201587;

and

hybridoma cell line LT003 (PD-1-14C12), which was preserved in China Center for Type Culture Collection (CCTCC) on June 16, 2015, with the CCTCC Deposit Accession

NO: C2015105.

The inventors surprisingly found that:

Hybridoma cell line LT002 is capable of secreting a specific monoclonal antibody (named 4G10) that binds specifically to CTLA4, and the monoclonal antibody can effectively block the interaction of CTLA4 to B7.

Hybridoma cell line LT003 is capable of secreting a specific monoclonal antibody (named 14C12) that specifically binds to PD-1, and the monoclonal antibody can effectively block the interaction of PD-1 to PDL1.

Furthermore, the inventors generated humanized antibodies against CTLA4 (named 4G10H1L1, 4G10H3L3, 4G10H4L3 and 8D2H14L2, respectively) and humanized antibodies against PD-1 (named 14C12H1L1) in a creative way.

Furthermore, the inventors created a series of new humanized bispecific antibodies (named BiAb001 BiAb002 BiAb003, BiAb004, BiAb007 and BiAb010, respectively) via recombining the two kinds of humanized antibodies, which can bind both CTLA4 and PD-1, and block interactions of CTLA4 with B7, and PD-1 with PDL1. The bispecific antibodies can effectively bind and activate human T cells, induce lymphocytes to secrete IFN-γ and IL-2, with the potential to be prepared into drugs for prevention and treatment of cancers, such as lung cancer, melanoma, renal cancer, ovarian cancer and leukemia.

The following are provided by the present disclosure:

In one aspect, there is provided a bispecific antibody thereof, wherein,

the first protein functional area targets PD-1,

and

the second protein functional area targets CTLA4.

In one embodiment of the disclosure, the said bispecific antibody, wherein the said first and second protein functional areas are connected directly or via connecting fragments; Preferably, the connecting fragments are (GGGGS)n, and n is a positive integer, such as 1, 2, 3, 4, 5 or 6.

In one embodiment of the disclosure, the said bispecific antibody, wherein,

the said first and second protein functional areas are respectively immunoglobulins

or their antigen-binding fragments, such as half antibody, Fab, F(ab')2 or single-chain antibody.

Preferably, the said first protein functional area is an immunoglobulin, and the said second protein functional area is a single-chain antibody;

or,

Preferably, the said first protein functional area is a single-chain antibody, and the said second protein functional area is an immunoglobulin.

In one embodiment of the disclosure, the said bispecific antibody, wherein, the quantity of the first protein functional area or the second protein functional area is one, two, or more, independently.

In one embodiment of the disclosure, the said bispecific antibody, wherein, the said immunoglobulin is IgG, IgA, IgD, IgE or IgM; Preferably IgG, such as IgG1, IgG2, IgG3, or IgG4.

In one embodiment of the disclosure, the said bispecific antibody, wherein, the said single-chain antibody is attached at the c-terminal of the heavy chain of the immunoglobulin. Since one immunoglobulin consists of two heavy chains, thus one immunoglobulin molecule is linked to two single-chain antibody molecules. Preferably, the two said single-chain antibody molecules are the same.

In one embodiment of the disclosure, the said bispecific antibody, wherein,

the heavy chain variable region of the said immunoglobulin comprises CDRs with the amino acid sequences of SEQ ID NO: 29-31, and the light chain variable region of the said immunoglobulin comprises CDRs with the amino acid sequences of SEQ ID NO: 32-34;

And/or,

the heavy chain variable region of the said single-chain antibody comprises CDRs with the amino acid sequences of SEQ ID NO: 35 – 37, or SEQ ID NO: 35, SEQ ID NO: 41 and SEQ ID NO: 37, or SEQ ID NO: 42-44; and the light chain variable region of the said single-chain antibody comprises CDRs with the amino acid sequences of SEQ ID NO: 38-40, or SEQ ID NO: 45-47.

In one embodiment of the disclosure, the said bispecific antibody, wherein,

the heavy chain variable region of the said immunoglobulin comprises CDRs with the amino acid sequences of SEQ ID NO: 35-37, or SEQ ID NO: 35, SEQ ID NO: 41 and SEQ ID NO: 37, or SEQ ID NO: 42-44; and the light chain variable region of the said immunoglobulin comprises CDRs with the amino acid sequences of SEQ ID NO: 38-40, or SEQ ID NO: 45-47;

and/or,

the heavy chain variable region of the said single-chain antibody comprises CDRs with the amino acid sequences of SEQ ID NO: 29-31; and the light chain variable region of the said single-chain antibody comprises CDRs with the amino acid sequences of SEQ ID NO: 32-34.

In one embodiment of the disclosure, the said bispecific antibody, wherein,

the amino acid sequence of the heavy chain variable region of the said immunoglobulin is selected from SEQ ID NO. 16 or SEQ ID NO. 20; the amino acid sequence of the light chain variable region of the said immunoglobulin is selected from SEQ ID NO: 18 or SEQ ID NO: 22;

And/or,

the amino acid sequence of the heavy chain variable region of the said single-chain antibody is selected from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14 or SEQ ID NO: 25; the amino acid sequence of the light chain variable region of the said single chain antibody is selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12 or SEQ ID NO: 27.

In one embodiment of the disclosure, the said bispecific antibody, wherein,

the amino acid sequence of the heavy chain variable region of the said immunoglobulin is selected from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14 or SEQ ID NO: 25; the amino acid sequence of the light chain variable region of the single-chain antibody is selected from SEQ ID NO: 4, SEQ ID NO:8, SEQ ID NO:12 or SEQ ID NO:27;

and/or,

The amino acid sequence of the heavy chain variable region of the said single-chain antibody is selected from SEQ ID NO: 16 or SEQ ID NO: 20; the amino acid sequence of

the light chain variable region of the said immunoglobulin is selected from SEQ ID NO: 18 or SEQ ID NO: 22.

In one embodiment of the disclosure, the said bispecific antibody, wherein,

the said immunoglobulin contains non-CDR regions from species other than mouse, for example, from human.

In one embodiment of the disclosure, the constant region of the said immunoglobulin is humanized. For example, the constant region of the heavy chain is Ig gamma-1 chain C region, ACCESSION: P01857; the constant region of light chain is Ig kappa chain C region, ACCESSION: P01834.

In one embodiment of the disclosure, the said bispecific antibody, wherein, the said bispecific antibody binds to CTLA4 protein and/or PD-1 protein with a 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 or 10^{-10} M or less.

The present disclosure also relates to a bispecific antibody, whose heavy chain variable region comprises CDRs with amino acid sequences of SEQ ID NO: 29-31, SEQ ID NO: 35-37, or SEQ ID NO: 35, SEQ ID NO: 41, and SEQ ID NO: 37, or SEQ ID NO: 42-44;

and,

of SEQ ID NO: 32-34, or SEQ ID NO: 38-40, or SEQ ID NO: 45-47;

And light chain variable region comprises CDRs with amino acid sequences of SEQ ID NO: 32-34, or SEQ ID NO: 38-40, or SEQ ID NO: 45-47;

Preferably, the CDRs in the light chain variable region and the heavy chain variable region are not the same.

Disclosed is an isolated nucleic acid molecule comprising a nucleotide sequence capable of encoding the heavy chain variable region of the antibody, wherein,

the heavy chain variable region of the said antibody comprises CDRs with amino acid sequences selected from SEQ ID NO: 29-31, SEQ ID NO: 35-37, or SEQ ID NO: 35, SEQ ID NO: 41, and SEQ ID NO: 37, or SEQ ID NO: 42-44;

and,

selected from SEQ ID NO: 32-34, or SEQ ID NO: 38-40, or SEQ ID NO: 45-47.

In another aspect, there is provided an isolated nucleic acid molecule comprising a nucleotide sequence capable of encoding a heavy chain variable region of an antibody, wherein,

the heavy chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NO: 29-31, SEQ ID NO: 35-37, or SEQ ID NO: 35, SEQ ID NO: 41, and SEQ ID NO: 37, or SEQ ID NO: 42-44;

And,

Amino acid sequences are selected from SEQ ID NO: 32-34, or from SEQ ID NO: 38-40, or from SEQ ID NO: 45-47.

Disclosed is an isolated nucleic acid molecule comprising a nucleotide sequence capable of encoding the light chain variable region of the antibody, wherein,

the light chain variable region of the said antibody comprises CDRs with amino acid sequences selected from SEQ ID NO: 32-34, or SEQ ID NO: 38-40, or SEQ ID NO: 45-47.

In another aspect, there is provided an isolated nucleic acid molecule comprising a nucleotide sequence capable of encoding the light chain variable region of the antibody, wherein,

the light chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NO: 32-34, or SEQ ID NO: 38-40, or SEQ ID NO: 45-47.

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

In another aspect, there is provided a vector, comprising the isolated nucleic acid molecule as described herein.

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

In another aspect, there is provided a host cell line, comprising the isolated nucleic acid molecule as described herein, or the vector as described herein.

Disclsoed is a method for preparing the bispecific antibodies described in the present disclosure, by culturing the host cells in the present disclosure under appropriate conditions, and recovering the said bispecific antibodies from the cell culture.

In another aspect, there is provided a method for preparing the bispecific antibodies thereof as described herein, by culturing the host cell line as described herein under appropriate conditions, and recovering the bispecific antibodies from the cell culture.

Disclosed are conjugates, including the bispecific antibodies described in the present disclosure and a conjugating partner as a detectable marker. Specifically, the said conjugating partners are radioactive isotopes, fluorescein, luminescent materials, colorful substances, or enzymes.

In another aspect, there is provided a conjugate, comprising the bispecific antibody as described herein and a conjugating partner as a detectable marker; Preferably, the conjugating partner is a radioactive isotope, fluorescein, a luminescent material, a colorful substance, or an enzyme.

Disclosed are reagent kits, consisting of the bispecific antibodies or the conjugates described in the disclosure.

In another aspect, there is provided a reagent kit, comprising the bispecific antibody as described herein, or the conjugate as described herein;

Preferably, the reagent kit may contain a secondary antibody, which specifically recognizes the bispecific antibody; optionally, such secondary antibody may contain a detectable marker, wherein the detectable marker is a radioactive isotope, fluorescein, a luminescent material, a colorful substance, or an enzyme.

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

Disclosed is usage of the said bispecific antibodies described in the present disclosure to prepare reagent kits for detection of the existence or the levels of CTLA4 and/or PD-1 in samples.

In another aspect, there is provided use of the bispecific antibody as described herein in the preparation of a kit for the detection of the existence or the levels of CTLA4 and/or PD-1 in a sample.

Disclosed is a pharmaceutical composition comprising the said

bispecific antibodies or the conjugates 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 bispecific antibody as described herein, or the conjugate as described herein; Optionally, the pharmaceutical compsition also comprises a pharmaceutically acceptable carrier or excipient.

Disclosed is use of the bispecific antibodies or conjugates described in the disclosure for producing drugs that are used for prevention and/or treatment of tumors or anemia, or for 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 bispecific antibody as described herein, or the conjugate as described herein, for the manufacturing of a medicament for prevention, treatment, adjuvant treatment, and/or diagnosis of tumors or anemia; preferably the tumors are selected from the group consisting of melanoma, renal cancer, prostate cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, liver cancer, non-small cell lung cancer, ovarian cancer and leukemia.

The present inventors have found through animal experiments that, bispecific antibody BiAb004 described in the disclosure can effectively inhibit the growth of MC38 tumor cells inoculated subcutaneously in right side of PD-1 HuGEMM mice, which the growth of tumor volume in PD-1 HuGEMM tumor-bearing mice is significantly inhibited.

Disclosed is the use of the bispecific antibodies or the conjugates described in the present disclosure, to prepare drugs with the following purposes:

Testing CTLA4 level in samples,

Blocking CTLA4 binding to B7,

Regulating (e.g. down-regulating) CTLA4 activity or CTLA4 levels,

Removing immunosuppression of CTLA4,

Activating T lymphocytes, or

Increasing the secretion of IL-2 in T lymphocytes;

And/or,

Blocking PD-1 binding to PDL1,

Regulating (e.g. down-regulating) PD-1 activity or PD-1 levels,

Removing immunosuppression of PD-1, or

Increasing the secretion of IFN-γ in T lymphocytes.

In another aspect, there is provided use of the bispecific antibody as described herein, or the conjugate as described herein, for the manufacturing of a medicament selected from the following:

a medicament for testing CTLA4 level in samples,

a medicament for blocking CTLA4 binding to B7,

a medicament for regulating (e.g. down-regulating) CTLA4 activity or CTLA4 levels,

a medicament for removing immunosuppression of CTLA4,

a medicament for activating T lymphocytes, or

a medicament for increasing the secretion of IL-2 in T lymphocytes;

And/or,

a medicament for blocking PD-1 binding to PDL1,

a medicament for regulating (e.g. down-regulating) PD-1 activity or PD-1 levels,

a medicament for removing immunosuppression of PD-1, or

a medicament for increasing the secretion of IFN-γ in T lymphocytes.

The present disclosure relates to an in vivo or in vitro method to apply to cells or subjects in need with an effective dose of the bispecific antibodies or the conjugates described in the present disclosure, and the said method is selected from the following:

Testing CTLA4 level in samples,

Blocking CTLA4 binding to B7,

Regulating (e.g. down-regulating) CTLA4 activity or CTLA4 levels,

Removing immunosuppression of CTLA4,

Activating T lymphocytes, or

Increasing the secretion of IL-2 in T lymphocytes;

And/or,

Blocking PD-1 binding to PDL1,

Regulating (e.g. down-regulating) PD-1 activity or PD-1 levels,

Removing immunosuppression of PD-1, or

Increasing the secretion of IFN-γ in T lymphocytes.

In another aspect, there is provided an in vivo or in vitro method, comprising administering to a cell or a subject in need with an effective dose of the bispecific antibody as described herein, or the conjugate as described herein, and for the purpose selected from the following:

Testing CTLA4 level in samples,

Blocking CTLA4 binding to B7,

Regulating (e.g. down-regulating) CTLA4 activity or CTLA4 levels,

Removing immunosuppression of CTLA4,

Activating T lymphocytes, or

Increasing the secretion of IL-2 in T lymphocytes;

And/or,

Blocking PD-1 binding to PDL1,

Regulating (e.g. down-regulating) PD-1 activity or PD-1 levels,

Removing immunosuppression of PD-1, or

Increasing the secretion of IFN-γ in T lymphocytes.

In in vitro experiments in the present disclosure, the anti-CTLA4 antibodies, the anti-PD-1 antibodies, and the anti-CTLA4-anti-PD-1 bispecific antibodies described in the present disclosure all can induce the secretion of IFN-γ, and activate the immune response.

Disclosed is a method for the prevention and/or treatment of tumors or anemia, or for diagnosis of tumors or anemia, including

procedures to apply to subjects in need with an effective dose of the bispecific antibodies or the conjugates 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 bispecific antibody as described herein, or the conjugate as described herein, for the manufacturing of a medicament for the prevention, treatment, adjuvant treatment, and/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 or leukemia.

The use of bispecific antibodies or conjugates thereof described in the present disclosure for the prevention and/or treatment of tumors or anemia, or for 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.

Use of bispecific antibodies or the conjugates thereof described in the present disclosure, with the following purposes:

Blocking CTLA4 binding to B7,

Regulating (e.g. down-regulating) CTLA4 activity or CTLA4 levels,

Claims

1. A bispecific antibody thereof, wherein,

the first protein functional area binds to PD-1,

And,

the second protein functional area binds to CTLA4.

2. The bispecific antibody thereof described in Claim 1, wherein,

the first protein functional area and the second protein functional area are directly connected or connected by a connecting fragment; Preferably, the connecting fragment is (GGGGS)n, and n is a positive integer, such as 1, 2, 3, 4, 5 or 6.

3. The bispecific antibody thereof described in Claims 1 or 2, wherein,

the first and second protein functional areas are individually immunoglobulins or their antigen-binding fragments, such as half antibody, Fab, F(ab')2 or single-chain antibody;

Preferably, the first protein functional area is an immunoglobulin, and the second protein functional area is a single-chain antibody;

Or,

Preferably, the first protein functional area is a single-chain antibody, and the second protein functional area is an immunoglobulin.

4. The bispecific antibody described in any one of Claims 1 to 3, wherein,

the quantity of the first protein functional area or the second protein functional area is one, two, or more, independently.

5. The bispecific antibody described in any one of Claims 3 or 4, wherein, the immunoglobulin is IgG, IgA, IgD, IgE or IgM; Preferably, the immunoglobulin is IgG, and optionally, the IgG is IgG1, IgG2, IgG3, or IgG4.

6. The bispecific antibody described in any one of Claims 3 to 5, wherein,

the single-chain antibody is attached at the c-terminal of the heavy chain of the

immunoglobulin.

7. The bispecific antibody described in any one of Claims 3 to 6, wherein, the bispecific antibody has the following features:

(1) the heavy chain variable region of the immunoglobulin comprises CDRs with the amino acid sequences of SEQ ID NO: 29-31, and the light chain variable region of the immunoglobulin comprises CDRs with the amino acid sequences of SEQ ID NO: 32-34;

And/or,

the heavy chain variable region of the single-chain antibody comprises CDRs with the amino acid sequences of SEQ ID NO: 35 – 37, or SEQ ID NO: 35, SEQ ID NO: 41 and SEQ ID NO: 37, or SEQ ID NO: 42-44; and the light chain variable region of the single-chain antibody comprises CDRs with the amino acid sequences of SEQ ID NO: 38-40, or SEQ ID NO: 45-47.

or,

(2) the heavy chain variable region of the immunoglobulin comprises CDRs with the amino acid sequences of SEQ ID NO: 35-37, or SEQ ID NO: 35, SEQ ID NO: 41 and SEQ ID NO: 37, or SEQ ID NO: 42-44; and the light chain variable region of the immunoglobulin comprises CDRs with the amino acid sequences of SEQ ID NO: 38-40, or SEQ ID NO: 45-47;

And/or,

the heavy chain variable region of the single-chain antibody comprises CDRs with the amino acid sequences of SEQ ID NO: 29-31; and the light chain variable region of the single-chain antibody comprises CDRs with the amino acid sequences of SEQ ID NO: 32-34.

8. The bispecific antibody described in any one of Claims 3 to 7, wherein, the bispecific antibody has the following features:

(1) the amino acid sequence of the heavy chain variable region of the immunoglobulin is selected from SEQ ID NO. 16 or SEQ ID NO. 20; the amino acid sequence of the light chain variable region of the immunoglobulin is selected from SEQ

ID NO: 18 or SEQ ID NO: 22;

And/or,

the amino acid sequence of the heavy chain variable region of the single-chain antibody is selected from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14 or SEQ ID NO: 25; the amino acid sequence of the light chain variable region of the single chain antibody is selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12 or SEQ ID NO: 27.

or,

(2) the amino acid sequence of the heavy chain variable region of the immunoglobulin is selected from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14 or SEQ ID NO: 25; the amino acid sequence of the light chain variable region of the single chain antibody is selected from SEQ ID NO: 4, SEQ ID NO:8, SEQ ID NO:12 or SEQ ID NO:27;

and/or,

the amino acid sequence of the heavy chain variable region of the single-chain antibody is selected from SEQ ID NO: 16 or SEQ ID NO: 20; the amino acid sequence of the light chain variable region of the immunoglobulin is selected from SEQ ID NO: 18 or SEQ ID NO: 22.

9. The bispecific antibody described in any one of Claims 1 to 8, wherein,

the immunoglobulin contains non-CDR regions from species other than mouse, and optionally, from human.

10. The bispecific antibody described in any one of Claims 1 to 9, wherein, the bispecific antibody binds to CTLA4 protein and/or PD-1 protein with a K_D less than approximately 10^{-5} M, and optionally, less than approximately 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M or less.

11. An isolated nucleic acid molecule comprising a nucleotide sequence capable of encoding a heavy chain variable region of an antibody, wherein,

the heavy chain variable region of the antibody comprises CDRs with amino acid

sequences selected from SEQ ID NO: 29-31, SEQ ID NO: 35-37, or SEQ ID NO: 35, SEQ ID NO: 41, and SEQ ID NO: 37, or SEQ ID NO: 42-44;

And,

Amino acid sequences are selected from SEQ ID NO: 32-34, or from SEQ ID NO: 38-40, or from SEQ ID NO: 45-47.

12. An isolated nucleic acid molecule comprising a nucleotide sequence capable of encoding the light chain variable region of the antibody, wherein,

the light chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NO: 32-34, or SEQ ID NO: 38-40, or SEQ ID NO: 45-47.

13. A vector, comprising the isolated nucleic acid molecule described in Claims 11 and/or 12.

14. A host cell line, comprising the isolated nucleic acid molecule described in Claims 11 and/or 12, or the vector described in Claim 13.

15. A method for preparing the bispecific antibodies thereof described in Claims 1 to 10, by culturing the host cell line in Claim 14 under appropriate conditions, and recovering the bispecific antibodies from the cell culture.

16. A conjugate, comprising the bispecific antibody described in any one of Claims 1 to 10 and a conjugating partner as a detectable marker; Preferably, the conjugating partner is a radioactive isotope, fluorescein, a luminescent material, a colorful substance, or an enzyme.

17. A reagent kit, comprising the bispecific antibody described in any one of Claims 1 to 10, or the conjugate described in Claim 16;

Preferably, the reagent kit may contain a secondary antibody, which specifically recognizes the bispecific antibody; optionally, such secondary antibody may contain a

detectable marker, wherein the detectable marker is a radioactive isotope, fluorescein, a luminescent material, a colorful substance, or an enzyme.

18. Use of the bispecific antibody of any one of Claims 1 to 10 in the preparation of a kit for the detection of the existence or the levels of CTLA4 and/or PD-1 in a sample.

19. A pharmaceutical composition, comprising the bispecific antibody of any one of Claims 1 to 10, or the conjugate of Claim 16; Optionally, the pharmaceutical compsition also comprises a pharmaceutically acceptable carrier or excipient.

20. Use of the bispecific antibody of any one of Claims 1 to 10, or the conjugate of Claim 16, for the manufacturing of a medicament for prevention, treatment, adjuvant treatment, and/or diagnosis of tumors or anemia; preferably the tumors are selected from the group consisting of melanoma, renal cancer, prostate cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, liver cancer, non-small cell lung cancer, ovarian cancer and leukemia.

21. Use of the bispecific antibody of any one of Claims 1 to 10, or the conjugate of Claim 16, for the manufacturing of a medicament selected from the following:

a medicament for testing CTLA4 level in samples,

a medicament for blocking CTLA4 binding to B7,

a medicament for regulating (e.g. down-regulating) CTLA4 activity or CTLA4 levels,

a medicament for removing immunosuppression of CTLA4,

a medicament for activating T lymphocytes, or

a medicament for increasing the secretion of IL-2 in T lymphocytes;

And/or,

a medicament for blocking PD-1 binding to PDL1,

a medicament for regulating (e.g. down-regulating) PD-1 activity or PD-1 levels,

a medicament for removing immunosuppression of PD-1, or

a medicament for increasing the secretion of IFN-γ in T lymphocytes.

22. An in vivo or in vitro method, comprising administering to a cell or a subject in need with an effective dose of the bispecific antibody of any one of Claims 1 to 10, or the conjugate of Claim 16, and for the purpose selected from the following:

Testing CTLA4 level in samples,

Blocking CTLA4 binding to B7,

Regulating (e.g. down-regulating) CTLA4 activity or CTLA4 levels,

Removing immunosuppression of CTLA4,

Activating T lymphocytes, or

Increasing the secretion of IL-2 in T lymphocytes;

And/or,

Blocking PD-1 binding to PDL1,

Regulating (e.g. down-regulating) PD-1 activity or PD-1 levels,

Removing immunosuppression of PD-1, or

Increasing the secretion of IFN-γ in T lymphocytes.

23. Use of the bispecific antibody of any one of Claims 1 to 10, or the conjugate of Claim 16, for the manufacturing of a medicament for the prevention, treatment, adjuvant treatment, and/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 or leukemia.

24. The bispecific antibody of any one of Claims 1 to 10, or the conjugate of Claim 16, for use in any one of the following:

Testing CTLA4 level in samples,

Blocking CTLA4 binding to B7,

Regulating (e.g. down-regulating) CTLA4 activity or CTLA4 levels,

Removing immunosuppression of CTLA4,

Activating T lymphocytes, or

Increasing the secretion of IL-2 in T lymphocytes;

And/or,

Blocking PD-1 binding to PDL1,

Regulating (e.g. down-regulating) PD-1 activity or PD-1 levels,

Removing immunosuppression of PD-1, or

Increasing the secretion of IFN-γ in T lymphocytes.

25. The use of claim 23, wherein an effective dose of the bispecific antibody or the conjugate is to be administered to the subject.