

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

In another aspect, the present disclosure refers to a composition comprising isolated nucleic acid molecules encoding for the bispecific antibody as disclosed herein, wherein the composition comprises a first isolated nucleic acid molecule comprising a nucleotide sequence encoding a heavy chain variable region of an antibody and a single-chain antibody, wherein

the heavy chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 29-31, and wherein the single-chain antibody comprises a heavy chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 35-37 and a light chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 38-40; and a second isolated nucleic acid molecule comprising a nucleotide sequence encoding the light chain variable region of an antibody, wherein, the light chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 32-34.

In another aspect, the present disclosure refers to a composition comprising isolated nucleic acid molecules encoding for the bispecific antibody as disclosed herein, wherein the composition comprises a first isolated nucleic acid molecule comprising a nucleotide sequence encoding a heavy chain variable region of an antibody and a single-chain antibody, wherein

the heavy chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 29-31,

and wherein the single-chain antibody comprises a heavy chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 35, 41 and 37 and a light chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 38-40; and a second isolated nucleic acid molecule comprising a nucleotide sequence encoding the light chain variable region of an antibody, wherein, the light chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 32-34.

In another aspect, the present disclosure refers to a composition comprising isolated nucleic acid molecules encoding for the bispecific antibody as disclosed herein, wherein the composition comprises a first isolated nucleic acid molecule comprising a nucleotide sequence encoding a heavy chain variable region of an antibody and a single-chain antibody, wherein

the heavy chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 29-31,

and wherein the single-chain antibody comprises a heavy chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 42-44 and a light chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 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; and a second isolated nucleic acid molecule comprising a nucleotide sequence encoding the light chain variable region of an antibody, wherein, the light chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 32-34.

In another example, the present disclosure refers to an isolated nucleic acid molecule comprising a nucleotide sequence encoding the light chain variable region of an antibody, wherein,

the light chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 32-34.

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

In another example, the present disclosure refers to a vector, comprising the isolated nucleic acid molecule as described herein or any combination thereof.

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, the present disclosure refers to a host cell line, comprising the composition as described herein.

Disclosed 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, the present disclosure refers to a method for preparing the bispecific antibody as described herein, the method comprising culturing the host cell line as described herein under appropriate conditions, and recovering the bispecific antibody 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, the present disclosure refers to a conjugate comprising the bispecific antibody as described herein and a conjugating partner as a detectable marker.

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

In another aspect, the present disclosure refers to a kit comprising the bispecific antibody as described herein, or the conjugate as described herein.

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 example, 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, the present disclosure refers to a pharmaceutical composition, comprising the bispecific antibody as described herein, or the conjugate as described herein.

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, the present disclosure refers to use of the bispecific antibody as described herein, or the conjugate as described herein, or the pharmaceutical composition as described herein for the manufacture of a medicament for the treatment of a tumor.

In another example, the present disclosure refers to use of the bispecific antibody as disclosed herein, the conjugate as disclosed herein or the pharmaceutical composition as disclosed herein for the manufacture of a medicament for the treatment of anemia.

In another aspect, the present disclosure refers to use of the bispecific antibody as disclosed herein, the conjugate as disclosed herein or the pharmaceutical composition as disclosed herein for the manufacture of a medicament for the treatment of a viral or parasitic infection.

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

Claims

1. A bispecific antibody comprising at least one first protein functional area that binds to PD-1 and at least one second protein functional area that binds to CTLA4, wherein the at least one first and at least one second protein functional areas each comprise an immunoglobulin or antigen-binding fragment thereof,

wherein the at least one first protein functional area comprises a heavy chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 29-31 and a light chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 32-34,

wherein the at least one second protein functional area comprises a heavy chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 35-37 and a light chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 38-40.

2. A bispecific antibody comprising at least one first protein functional area that binds to PD-1 and at least one second protein functional area that binds to CTLA4, wherein the at least one first and at least one second protein functional areas each comprise an immunoglobulin or antigen-binding fragment thereof,

wherein the at least one first protein functional area comprises a heavy chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 29-31 and a light chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 32-34,

wherein the at least one second protein functional area comprises comprising a heavy chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 35, 41 and 37 and a light chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 38-40.

3. A bispecific antibody comprising at least one first protein functional area that binds to PD-1 and at least one second protein functional area that binds to CTLA4, wherein the

at least one first and at least one second protein functional areas each comprise an immunoglobulin or antigen-binding fragment thereof,

wherein the at least one first protein functional area comprises a heavy chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 29-31 and a light chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 32-34,

wherein the at least one second protein functional area comprises a heavy chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 42-44 and a light chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 45-47.

4. The bispecific antibody of any one of claims 1-3, wherein the heavy chain variable region of the at least one first protein functional area comprises the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 20 and the light chain variable region of the at least one first protein functional area comprises the amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 22.

5. The bispecific antibody of claim 1, wherein the heavy chain variable region of the at least one second protein functional area comprises the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 6 or SEQ ID NO: 10 and light chain variable region of the at least one second protein functional area comprises the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 8 or SEQ ID NO: 12.

6. The bispecific antibody of claim 2, wherein the heavy chain variable region of the at least one second protein functional area comprises the amino acid sequence of SEQ ID NO: 14 and light chain variable region of the at least one second protein functional area comprises the amino acid sequence of SEQ ID NO: 12.

7. The bispecific antibody of claim 3, wherein the heavy chain variable region of the at least one second protein functional area comprises the amino acid sequence of SEQ ID

NO: 25 and light chain variable region of the at least one second protein functional area comprises the amino acid sequence of SEQ ID NO: 27.

8. The bispecific antibody of any one of claims 1-7, wherein,
the antigen-binding fragment comprises a half antibody, a Fab, a F(ab')₂ or a single-chain antibody.
9. The bispecific antibody of any one of claims 1-8, wherein the at least one first protein functional area comprises an immunoglobulin and the at least one second protein functional area comprises a single-chain antibody.
10. The bispecific antibody of any one of claims 1-8, wherein the at least one first protein functional area comprises a single-chain antibody and the at least one second protein functional area comprises an immunoglobulin.
11. The bispecific antibody of any one of claims 1-10, wherein the bispecific antibody comprises at least two or more first protein functional areas, at least two or more second protein functional areas or any combination thereof.
12. The bispecific antibody of any one of claims 1-11, wherein the immunoglobulin is IgG, IgA, IgD, IgE or IgM.
13. The bispecific antibody of claim 12, wherein the immunoglobulin is IgG.
14. The bispecific antibody of claim 13, wherein the immunoglobulin is IgG1, IgG2, IgG3 or IgG4.
15. The bispecific antibody of any one of claims 9-14, wherein the single-chain antibody is attached at the c-terminus of the heavy chain of the immunoglobulin.

16. The bispecific antibody of any one of claims 1-15, wherein the immunoglobulin or antigen-binding fragment thereof contains non-CDR regions from species other than mouse, and optionally, from human.

17. The bispecific antibody of any one of claims 1-16, 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.

18. A composition comprising isolated nucleic acid molecules encoding for the bispecific antibody of claim 1, wherein the composition comprises

a first isolated nucleic acid molecule comprising a nucleotide sequence encoding a heavy chain variable region of an antibody and a single-chain antibody, wherein

the heavy chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 29-31,

and wherein the single-chain antibody comprises a heavy chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 35-37 and a light chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 38-40; and

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

the light chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 32-34.

19. A composition comprising isolated nucleic acid molecules encoding for the bispecific antibody of claim 2, wherein the composition comprises

a first isolated nucleic acid molecule comprising a nucleotide sequence encoding a heavy chain variable region of an antibody and a single-chain antibody, wherein

the heavy chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 29-31,

and wherein the single-chain antibody comprises a heavy chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 35, 41 and 37 and a

light chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 38-40; and

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

the light chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 32-34.

20. A composition comprising isolated nucleic acid molecules encoding for the bispecific antibody of claim 3, wherein the composition comprises

a first isolated nucleic acid molecule comprising a nucleotide sequence encoding a heavy chain variable region of an antibody and a single-chain antibody, wherein

the heavy chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 29-31,

and wherein the single-chain antibody comprises a heavy chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 42-44 and a light chain variable region comprising CDRs with the amino acid sequences of SEQ ID NOs: 45-47; and

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

the light chain variable region of the antibody comprises CDRs with amino acid sequences selected from SEQ ID NOs: 32-34.

21. The composition of any one of claims 18-20, wherein the first isolated nucleic molecule and the second isolated nucleic molecule are present in a single vector.

22. The composition of any one of claims 18-20, wherein the first isolated nucleic molecule is present in a first vector and the second isolated nucleic molecule are present in a second vector.

23. A host cell line, comprising the composition of any one of claims 18-22.

24. A method for preparing the bispecific antibody of any one of claims 1-17, the method comprising culturing the host cell line of claim 23 under appropriate conditions, and recovering the bispecific antibody from the cell culture.
25. A conjugate comprising the bispecific antibody of any one of claims 1-17 and a conjugating partner as a detectable marker.
26. The conjugate of claim 25, wherein the conjugating partner is a radioactive isotope, fluorescein, a luminescent material, a colorful substance, or an enzyme.
27. A kit comprising the bispecific antibody of any one of claims 1-17 or the conjugate of any one of claims 25-26.
28. The kit of claim 27, wherein the kit further comprises a secondary antibody, wherein the secondary antibody specifically recognizes the bispecific antibody.
29. The kit of claim 28, wherein the secondary antibody comprises a detectable marker.
30. The kit of claim 29, wherein the detectable marker is a radioactive isotope, fluorescein, a luminescent material, a colorful substance, or an enzyme.
31. A pharmaceutical composition, comprising the bispecific antibody of any one of claims 1-17 or the conjugate of any one of claims 25-26.
32. The pharmaceutical composition of claim 31, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier or excipient.
33. Use of the bispecific antibody of any one of claims 1-17, the conjugate of any one of claims 25-26 or the pharmaceutical composition of any one of claims 31-32 for the manufacture of a medicament for the treatment of a tumor.

34. The use of claim 33, wherein the tumor is 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.

35. Use of the bispecific antibody of any one of claims 1-17, the conjugate of any one of claims 25-26 or the pharmaceutical composition of any one of claims 31-32 for the manufacture of a medicament for the treatment of a viral or parasitic infection.

36. The use of any one of claims 33-35, wherein the bispecific antibody or the conjugate is to be administered to the subject in at least one therapeutically effective amount.