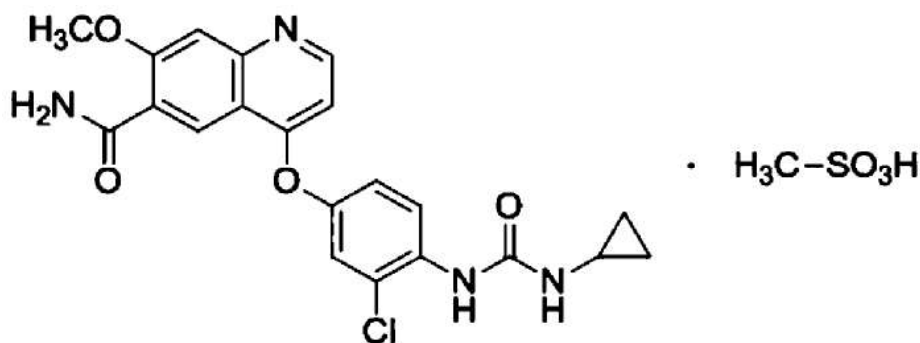




formula II

However, for a variety of tumors, the disease is still uncontrollable for a long term after chemotherapy, and the 5-year survival rate is still very low. Therefore, developing a medication or combination therapy with lower toxicity and higher efficacy is of great meaning.

## SUMMARY

By intensive research and creative efforts, the inventor correspondingly modified the Fc fragment of the anti-CTLA4/anti-PD-1 antibody structure to reduce the binding capacity of the Fc region to Fc receptors, thereby reducing ADCC, ADCP and/or CDC effects on immune cells and increasing the efficacy of the anti-CTLA4/anti-PD-1 antibody.

The present ~~invention~~disclosure is detailed below.

One aspect of the present ~~invention~~disclosure relates to a bispecific antibody, comprising:

a first protein functional region targeting PD-1, and

a second protein functional region targeting CTLA4;

wherein the first protein functional region is an immunoglobulin, and the second protein functional region is a single chain antibody; or, the first protein functional region is a single chain antibody, and the second protein functional region is an immunoglobulin;

wherein,

for the immunoglobulin, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 27–29 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 30–32 respectively; for the single chain antibody, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 33–35 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 36–38 respectively;

or,

for the immunoglobulin, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 33–35 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 36–38 respectively; for the single chain antibody, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 27–29 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 30–32 respectively;

the immunoglobulin is of human IgG1 subtype;

wherein, according to the EU numbering system, the heavy chain constant region of the immunoglobulin has mutations at any 2 or 3 of positions 234, 235 and 237, and the affinity constant of the bispecific antibody to FcγRIIIa and/or C1q is reduced after the mutation as compared to that before the mutation; preferably, the affinity constant is measured by a Fortebio Octet system.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the bispecific antibody, after the mutation described above, the affinity constant of the bispecific antibody to FcγRIIIa, FcγRI, FcγRIIa\_H131, FcγRIIIa\_V158 and/or FcγRIIb is reduced as compared to that before the mutation; preferably, the affinity constant is measured by a Fortebio Octet system.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the bispecific antibody, according to the EU numbering system, the heavy chain constant region of the immunoglobulin has the following mutations at positions 234, 235 and/or 237:

L234A and L235A;

L234A and G237A;

L235A and G237A;

or

L234A, L235A and G237A.

In the present **inventiondisclosure**, letters before the position number represent amino acids before mutation, and letters after the position number represent amino acids after mutation, unless otherwise specified.

**In another aspect,** ~~the~~ The present **inventiondisclosure** further relates to a bispecific antibody, comprising:

a first protein functional region targeting PD-1, and

a second protein functional region targeting CTLA4;

wherein the first protein functional region is an immunoglobulin, and the second protein functional region is a single chain antibody; or, the first protein functional region is a single chain antibody, and the second protein functional region is an immunoglobulin;

wherein,

for the immunoglobulin, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 27–29 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 30–32 respectively; for the single chain antibody, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 33–35 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 36–38 respectively;

or,

for the immunoglobulin, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 33–35 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 36–38 respectively; for the single chain antibody, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 27–29 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 30–32 respectively;

the immunoglobulin is of human IgG1 subtype;

wherein, according to the EU numbering system, the heavy chain constant region of the immunoglobulin has the following mutations at positions 234, 235 and/or 237:

L234A and L235A;

L234A and G237A;

L235A and G237A; or

L234A, L235A and G237A.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the bispecific antibody, according to the EU numbering system, the heavy chain constant region of the immunoglobulin has one or more mutations selected from:

N297A, D265A, D270A, P238D, L328E, E233D, H268D, P271G, A330R, C226S, C229S, E233P, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, N297Q, P238S, P238A, A327Q, A327G, P329A, K322A, T394D, G236R, G236A, L328R, A330S, P331S, H268A, E318A and K320A.

In one or more **embodimentexamples** of the present **inventiondisclosure**, the bispecific antibody is in the form of IgG-scFv, i.e., the Morrison format.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the bispecific antibody,

the amino acid sequence of the heavy chain variable region of the immunoglobulin is selected from SEQ ID NO: 14 and SEQ ID NO: 18; the amino acid sequence of the light

chain variable region of the immunoglobulin is selected from SEQ ID NO: 16 and SEQ ID NO: 20; 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: 41 and SEQ ID NO: 43; 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, SEQ ID NO: 42 and SEQ ID NO: 44;

or,

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: 41 and SEQ ID NO: 43; the amino acid sequence of the light chain variable region of the immunoglobulin is selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 42 and SEQ ID NO: 44; the amino acid sequence of the heavy chain variable region of the single chain antibody is selected from SEQ ID NO: 14 and SEQ ID NO: 18; the amino acid sequence of the light chain variable region of the single chain antibody is selected from SEQ ID NO: 16 and SEQ ID NO: 20.

In one or more **embodiment examples** of the present **invention disclosure**, the bispecific antibody is selected from any one of the following (1)–(20):

(1)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 16; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 2, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 4;

(2)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 16; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 6, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 8;

(3)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 16; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 12;

the immunoglobulin is set forth in SEQ ID NO: 42; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 18, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 20;

and,

(20)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 43, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 44; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 18, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 20.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the bispecific antibody,

the amino acid sequence of the heavy chain of the immunoglobulin is set forth in SEQ ID NO: 40, and the amino acid sequence of the light chain of the immunoglobulin is set forth in SEQ ID NO: 24.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the bispecific antibody, the immunoglobulin or the antigen-binding fragment thereof binds to FcγRIIIa\_F158, FcγRI, FcγRIIa\_H131, FcγRIIIa\_V158 and/or FcγRIIb with an affinity constant greater than about  $10^{-7}$  M, for example, greater than about  $10^{-6}$  M,  $10^{-5}$  M,  $10^{-4}$  M, or  $10^{-3}$  M or greater; preferably, the affinity constant is measured by a Fortebio Octet system;

preferably, the immunoglobulin or the antigen binding fragment thereof has no binding signal or a binding signal of less than 0.1 nm to FcγRIIIa\_F158, FcγRI, FcγRIIa\_H131, FcγRIIIa\_V158 and/or FcγRIIb; preferably, the binding signal refers to a response measured by a Fortebio Octet system.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the bispecific antibody, the immunoglobulin or the antigen-binding fragment thereof binds to C1q with an affinity constant greater than about  $10^{-9}$  M, for example, greater than about  $10^{-8}$  M,  $10^{-7}$  M,  $10^{-6}$  M, or  $10^{-5}$  M or greater; preferably, the affinity constant is measured by a Fortebio Octet system;

preferably, the immunoglobulin or the antigen binding fragment thereof has no binding signal or a binding signal of less than 0.1 nm to C1q; preferably, the binding signal refers to a response measured by a Fortebio Octet system.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the bispecific antibody, the first protein functional region is linked to the second protein functional region either directly or via a linker fragment; and/or the heavy chain variable region of the single chain antibody is linked to the light chain variable region of the single chain antibody either directly or via a linker fragment.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the bispecific antibody, the linker fragment is (GGGS)<sub>n</sub>, n being a positive integer; preferably, n is 1, 2, 3, 4, 5 or 6.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the bispecific antibody, the numbers of the first protein functional region and the second protein functional region are each independently 1, 2 or more.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the bispecific antibody, the number of the first protein functional region is 1 and the number of the second protein functional region is 2.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the bispecific antibody, the single chain antibody is linked to the C terminus of the heavy chain of the immunoglobulin. Since an immunoglobulin has two heavy chains, two single chain antibody molecules are linked to one immunoglobulin molecule. Preferably, the two single chain antibody molecules are identical. Preferably, the single chain antibody is linked to the C terminus of the heavy chain of the immunoglobulin by forming an amide bond via the aforementioned linker fragment.

In one or more **embodimentexamples** of the present **inventiondisclosure**, the constant regions of the immunoglobulin are humanized. For example, the heavy chain constant region is Ig gamma-1 chain C region, ACCESSION: P01857, and the light chain constant region is Ig kappa chain C region, ACCESSION: P01834.

In one or more **embodimentexamples** of the present **inventiondisclosure**, the bispecific antibody binds to CTLA4 protein and/or PD-1 protein with a  $K_D$  less than about  $10^{-5}$  M, e.g., less than about  $10^{-6}$  M,  $10^{-7}$  M,  $10^{-8}$  M,  $10^{-9}$  M or  $10^{-10}$  M or less.

In one or more **embodimentexamples** of the present **inventiondisclosure**, the bispecific antibody is a monoclonal antibody.

In one or more **embodimentexamples** of the present **inventiondisclosure**, the bispecific antibody is a humanized antibody.

In ~~one or more embodiments~~ another aspect of the present **inventiondisclosure**, the bispecific antibody comprises:

a first protein functional region targeting PD-1, and

a second protein functional region targeting CTLA4;

the number of the first protein functional region is 1, and the number of the second protein functional region is 2;

wherein the first protein functional region is an immunoglobulin, and the second protein functional region is a single chain antibody;

the amino acid sequence of the heavy chain of the immunoglobulin is set forth in SEQ ID NO: 40, and the amino acid sequence of the light chain of the immunoglobulin is set forth in SEQ ID NO: 24;

the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 43, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 44;

the single chain antibody is linked to the C terminus of the heavy chain of the immunoglobulin;

the first protein functional region is linked to the second protein functional region via a first linker fragment; and the heavy chain variable region of the single chain antibody is linked to the light chain variable region of the single chain antibody via a second linker fragment; the first linker fragment and the second linker fragment are the same or different;

preferably, the amino acid sequences of the first linker fragment and second linker fragment are independently selected from SEQ ID NO: 25 and SEQ ID NO: 26;

preferably, the amino acid sequences of the first linker fragment and second linker fragments are set forth in SEQ ID NO: 26.

Another aspect of the present ~~invention~~disclosure relates to an isolated nucleic acid molecule encoding the bispecific antibody ~~according to any embodiment of the present invention~~as described herein.

In another aspect, ~~t~~The present ~~invention~~disclosure also relates to a vector comprising the isolated nucleic acid molecule of the present ~~invention~~disclosure.

In yet another aspect, ~~t~~The present ~~invention~~disclosure also relates to a host cell comprising the isolated nucleic acid molecule of the present ~~invention~~disclosure or the vector of the present ~~invention~~disclosure.

Another aspect of the present ~~invention~~disclosure relates to a conjugate comprising an antibody or antigen-binding fragment thereof, and a conjugated moiety, wherein the immunoglobulin is the bispecific antibody ~~according to any embodiment~~example of the present invention~~as described herein~~, and the conjugated moiety is a detectable label; preferably, the conjugated moiety is a radioisotope, a fluorescent substance, a luminescent substance, a colored substance, or an enzyme.

Another aspect of the present ~~invention~~disclosure relates to a kit comprising the bispecific antibody ~~according to any embodiment of the present invention~~as described herein or comprising the conjugate of the present ~~invention~~disclosure;

wherein preferably, the kit further comprises a second antibody capable of specifically recognizing the immunoglobulin or the antigen binding fragment thereof; optionally, the second antibody further comprises a detectable label, for example, a radioisotope, a fluorescent substance, a luminescent substance, a colored substance, or an enzyme.

Another aspect of the present ~~invention~~disclosure relates to use of the bispecific antibody or the conjugate ~~according to any embodiment of the present invention~~as described herein in preparing a kit for detecting the presence or level of PD-1 and/or CTLA4 in a sample.

Another aspect of the present ~~invention~~disclosure relates to a pharmaceutical composition comprising the bispecific antibody or the conjugate ~~as described herein~~according to any embodiment of the present invention; optionally, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier and/or excipient.

In one or more **embodimentexamples** of the present **inventiondisclosure**, the pharmaceutical composition further comprises one or more anti-tumor chemotherapeutics;

preferably, the anti-tumor chemotherapeutic is a tyrosine kinase inhibitor; more preferably, the anti-tumor chemotherapeutic is anlotinib or a pharmaceutically acceptable salt thereof (e.g., hydrochloride salt), or lenvatinib or a pharmaceutically acceptable salt thereof (e.g., mesylate salt).

In one or more **embodimentexamples** of the present **inventiondisclosure**, the unit dose of the pharmaceutical composition is 100–1000 mg, 200–800 mg, 200–500 mg, 300–600 mg, 400–500 mg, or 450 mg, based on the mass of the bispecific antibody.

Another aspect of the present **inventiondisclosure** relates to a combination product comprising a first product and a second product in separate packages,

wherein,

the first product comprises the bispecific antibody, the conjugate or the pharmaceutical composition ~~according to any embodiment of the present invention~~ as described herein;

the second product comprises one or more anti-tumor chemotherapeutics; preferably, the anti-tumor chemotherapeutic is a tyrosine kinase inhibitor; more preferably, the anti-tumor chemotherapeutic is anlotinib or a pharmaceutically acceptable salt thereof (e.g., hydrochloride salt), or lenvatinib or a pharmaceutically acceptable salt thereof (e.g., mesylate salt);

preferably, the first product and the second product further independently comprise one or more pharmaceutically acceptable excipients;

preferably, the combination product further comprises a package insert.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the combination product, the unit dose of the first product is 100–1000 mg, 200–800 mg, 200–500 mg, 300–600 mg, 400–500 mg, or 450 mg, based on the mass of the bispecific antibody.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the combination product, the unit dose of the second product is 0.1–100 mg, 0.5–50 mg, 0.5–10 mg, 1–10 mg, 2–8 mg, or 1–5 mg, based on the mass of the active ingredient.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the combination product, the unit dose of the second product is 1–20 mg, 2–15 mg, 4–12 mg, or 8–12 mg, based on the mass of the active ingredient.

In a further aspect, the present disclosure relates to use of the bispecific antibody as described herein or the conjugate as described herein in preparing a medicament for treating and/or preventing a tumor or anemia, or in preparing a medicament for diagnosing

a tumor or anemia;

preferably, the tumor is selected from one or more of melanoma, renal cancer, prostate cancer, bladder cancer, colon cancer, rectal cancer, gastric cancer, liver cancer, lung cancer, ovarian cancer, leukemia, breast cancer, mesothelioma, cervical cancer, endometrial cancer, lymphoma and nasopharyngeal cancer;

preferably, the lung cancer is selected from one or more of non-small cell lung cancer, small cell lung cancer and squamous cell lung cancer;

preferably, the gastric cancer is gastric adenocarcinoma or gastroesophageal junction adenocarcinoma; preferably, the tumor is a solid tumor of MSI-H/dMMR phenotype; preferably, the tumor is selected from one or more of the following tumors of MSI-H/dMMR phenotype:

colon cancer, rectal cancer, endometrial cancer, gastric cancer, mesothelioma, sarcoma, adrenocortical carcinoma, malignant melanoma and ovarian germ cell neoplasm.

Another ~~aspect~~example of the present ~~invention~~disclosure relates to use of the bispecific antibody, the conjugate, the pharmaceutical composition or the combination product according to any ~~embodiment~~example of the present ~~invention~~disclosure in preparing a medicament for treating and/or preventing a tumor or anemia, or in preparing a medicament for diagnosing a tumor or anemia; preferably, the tumor is selected from one or more of melanoma, renal cancer,

next-generation sequencing, or NGS) can also be used as a method for detecting microsatellite instability. When more microsatellite loci are selected, such as more than 5 loci or other microsatellite loci, for PCR assay, inconsistency in  $\geq 30\%$  loci is defined as MSI-H, consistency in all loci is defined as MSS, and inconsistency between 0 and 30% is defined as MSI-L.

Another aspect of the present **inventiondisclosure** relates to use of the bispecific antibody or the conjugate ~~according to any embodiment of the present invention~~as described herein in preparing:

a medicament for blocking the binding of PD-1 to PD-L1,

a medicament for down-regulating the activity or level of PD-1,

a medicament for relieving the immunosuppression of PD-1 in an organism, or

a medicament for elevating IFN- $\gamma$  and/or IL-2 expression in T lymphocytes;

and/or

a medicament for blocking the binding of CTLA4 to B7,

a medicament for down-regulating the activity or level of CTLA4,

a medicament for relieving the immunosuppression of CTLA4 in an organism, or

a medicament for elevating IL-2 expression in T lymphocytes.

Another **aspectexample** of the present **inventiondisclosure** relates to a method for the prevention and/or treatment and/or adjuvant treatment and/or diagnosis of a tumor, comprising: administering to a subject in need an effective amount of the bispecific antibody, the conjugate, the pharmaceutical composition or the combination product according to any **embodimentexample** of the present **inventiondisclosure**; preferably, the tumor is selected from one or more of melanoma, renal cancer, prostate cancer, bladder cancer, colon cancer, rectal cancer, gastric cancer, liver cancer, lung cancer, ovarian cancer, leukemia, breast cancer, mesothelioma, cervical cancer, endometrial cancer, lymphoma and nasopharyngeal cancer;

preferably, the lung cancer is selected from one or more of non-small cell lung cancer, small cell lung cancer and squamous cell lung cancer;

preferably, the gastric cancer is gastric adenocarcinoma or gastroesophageal junction adenocarcinoma;

preferably, the tumor is a solid tumor of MSI-H/dMMR phenotype; preferably, the tumor is selected from one or more of the following tumors of MSI-H/dMMR phenotype:

colon cancer, rectal cancer, endometrial cancer, gastric cancer, mesothelioma, sarcoma, adrenocortical carcinoma, malignant melanoma and ovarian germ cell neoplasm.

In one or more **embodimentexamples** of the present **inventiondisclosure**, for the method, the administration is before or after a surgical treatment and/or before or after a radiotherapy.

In one or more **embodimentexample**s of the present **inventiondisclosure**, the method, wherein, the unit dose of anti-CTLA4/anti-PD-1 bispecific antibody is 0.1–100 mg, preferably 1–10 mg (e.g., 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg or 10 mg) per kg body weight; alternatively, the unit dose of the anti-CTLA4/anti-PD-1 bispecific antibody is 10–1000 mg (e.g., about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg or about 1000 mg), preferably 50–500 mg, 100–400 mg, 150–300 mg, 150–250 mg or 200 mg in each subject;

preferably, the dose is given once every 3 days, 4 days, 5 days, 6 days, 10 days, 1 week, 2 weeks or 3 weeks;

preferably, the route of administration is intravenous drip infusion or intravenous injection.

In some **embodimentexample**s, the administration of the anti-CTLA4/anti-PD-1 bispecific antibody is performed in cycles of 2 weeks (14 days) or 3 weeks (21 days), and preferably, the anti-CTLA4/anti-PD-1 bispecific antibody is administered intravenously on the first day (D1) of each cycle. For example, the anti-CTLA4/anti-PD-1 bispecific antibody is administered once every two weeks (q2w) or three weeks (q3w).

Also provided is the bispecific antibody, the conjugate, the pharmaceutical composition or the combination product according to any **embodimentexample** of the present **inventiondisclosure** for use in the prevention and/or treatment and/or adjuvant treatment and/or diagnosis of a tumor; preferably, the tumor is selected from one or more of melanoma, renal cancer, prostate cancer, bladder cancer, colon cancer, rectal cancer, gastric cancer, liver cancer, lung cancer, ovarian cancer, leukemia, breast cancer, mesothelioma, cervical cancer, endometrial cancer, lymphoma and nasopharyngeal cancer;

preferably, the lung cancer is selected from one or more of non-small cell lung cancer, small cell lung cancer and squamous cell lung cancer;

preferably, the gastric cancer is gastric adenocarcinoma or gastroesophageal junction adenocarcinoma;

preferably, the tumor is a solid tumor of MSI-H/dMMR phenotype; preferably, the tumor is selected from one or more of the following tumors of MSI-H/dMMR phenotype:

colon cancer, rectal cancer, endometrial cancer, gastric cancer, mesothelioma, sarcoma, adrenocortical carcinoma, malignant melanoma and ovarian germ cell neoplasm.

Provided is the bispecific antibody or conjugate according to any **embodimentexample** of the present **inventiondisclosure** for use in:

blocking the binding of PD-1 to PD-L1,

down-regulating the activity or level of PD-1,

**(9) BiAb004(M)**

The amino acid sequences of the 9 CDR regions associated with the heavy chain variable region are the same as BiAb001(M).

The amino acid sequences of the 3 CDR regions associated with the light chain variable region are the same as BiAb001(M).

For the antibody BiAb004(hG1TM) of the present **inventiondisclosure**, amino acid mutations are introduced into the non-variable region of BiAb004(M). According to the EU numbering system, amino acid mutations are introduced at positions 234, 235 and 237:

BiAb004(hG1TM) is obtained by introducing a leucine-to-alanine point mutation at position 234 (L234A), a leucine-to-alanine point mutation at position 235 (L235A), and a glycine-to-alanine point mutation at position 237 (G237A) in the hinge region of the heavy chain.

In the present **inventiondisclosure**, unless otherwise defined, the scientific and technical terms used herein have the meanings generally understood by those skilled in the art. In addition, the laboratory operations of cell culture, molecular genetics, nucleic acid chemistry and immunology used herein are the routine procedures widely used in the corresponding fields. Meanwhile, in order to better understand the present **inventiondisclosure**, the definitions and explanations of the relevant terms are provided below.

As used herein, when referring to the amino acid sequence of CTLA4 protein (cytotoxic T-lymphocyte antigen 4), it includes the full length of CTLA4 protein, or the extracellular fragment CTLA4ECD of CTLA4 or a fragment comprising CTLA4ECD; also included are fusion proteins of CTLA4ECD, such as a fragment fused to an Fc protein fragment of a mouse or human IgG (mFc or hFc). However, those skilled in the art will appreciate that in the amino acid sequence of the CTLA4 protein, mutations or variations (including but not limited to, substitutions, deletions and/or additions) can be naturally produced or artificially introduced without affecting biological functions thereof. Therefore, in the present **inventiondisclosure**, the term "CTLA4 protein" should include all such sequences, including their natural or artificial variants. In addition, when describing the sequence fragment of the CTLA4 protein, it also includes the corresponding sequence fragments in its natural or artificial variants.

As used herein, when referring to the amino acid sequence of PD-1 protein (NCBI GenBank: NM\_005018), it includes the full length of the PD-1 protein, or the extracellular fragment PD-1ECD of PD-1 or a fragment comprising PD-1ECD; also included are fusion proteins of PD-1ECD, such as a fragment fused to an Fc protein fragment of a mouse or human IgG (mFc or hFc). However, those skilled in the art will appreciate that in the amino acid sequence of the PD-1 protein, mutations or variations (including but not limited to, substitutions, deletions and/or additions) can be naturally produced or artificially introduced without affecting biological functions thereof. Therefore, in the present

**inventiondisclosure**, the term "PD-1 protein" should include all such sequences, including their natural or artificial variants. In addition, when describing the sequence fragment of the PD-1 protein, it also includes the corresponding sequence fragments in its natural or artificial variants.

As used herein, unless otherwise specified, the B7 is B7-1 and/or B7-2; specific sequences thereof are those known in the prior art, and reference may be made to sequences disclosed in the existing literature or GenBank. For example, B7-1 (CD80, NCBI Gene ID: 941); B7-2 (CD86, NCBI Gene ID: 942).

As used herein, the term EC<sub>50</sub> refers to the concentration for 50% of maximal effect, i.e., the concentration that can cause 50% of the maximal effect.

As used herein, the term "antibody" refers to an immunoglobulin molecule that generally consists of two pairs of polypeptide chains (each pair with one "light" (L) chain and one "heavy" (H) chain). In a general sense, the heavy chain can be interpreted as a polypeptide chain with a larger molecular weight in an antibody, and the light chain refers to a polypeptide chain with a smaller molecular weight in an antibody. Light chains are classified as  $\kappa$  and  $\lambda$  light chains. Heavy chains are generally classified as  $\mu$ ,  $\delta$ ,  $\gamma$ ,  $\alpha$ , or  $\epsilon$ , and isotypes of antibodies are defined as IgM, IgD, IgG, IgA, and IgE, respectively. In light chains and heavy chains, the variable region and constant region are linked by a "J" region of about 12 or more amino acids, and the heavy chain also comprises a "D" region of about 3 or more amino acids. Each heavy chain consists of a heavy chain variable region (V<sub>H</sub>) and a heavy chain constant region (C<sub>H</sub>). The heavy chain constant region consists of 3 domains (C<sub>H1</sub>, C<sub>H2</sub>, and C<sub>H3</sub>). Each light chain consists of a light chain variable region (V<sub>L</sub>) and a light chain constant region (C<sub>L</sub>). The light chain constant region consists of one domain C<sub>L</sub>. The constant region of the antibody can mediate the binding of immunoglobulins to host tissues or factors, including the binding of various cells of the immune system (e.g., effector cells) to the first component (C1q) of classical complement system. The V<sub>H</sub> and V<sub>L</sub> regions can be further subdivided into highly variable regions (called Complementarity Determining Regions (CDRs)), between which conservative regions called framework regions (FRs) are distributed. Each V<sub>H</sub> and V<sub>L</sub> consists of 3 CDRs and 4 FRs arranged from amino terminus to carboxyl terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions (V<sub>H</sub> and V<sub>L</sub>) of each heavy chain/light chain pair form antibody binding sites, respectively. The assignment of amino acids to each region or domain follows the definition of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, MD. (1987 and 1991)), Chothia & Lesk, (1987) *J. Mol. Biol.*, 196:901-917, or Chothia et al. (1989) *Nature*, 342:878-883. In particular, the heavy chain may also comprise more than 3 CDRs, such as 6, 9, or 12. For example, in the bispecific antibody of the present **inventiondisclosure**, the heavy chain may be an scFv with the C terminus of the heavy chain of IgG antibody linked to another antibody, and in this case, the heavy chain comprises 9 CDRs. The term "antibody" is not limited by any specific method for producing antibody. For example, the antibody includes, in particular, a

some embodiment examples, an antibody that specifically binds to an antigen (or an antibody that is specific for an antigen) refers to that the antibody binds to the antigen with an affinity ( $K_D$ ) of less than about  $10^{-5}$  M, such as less than about  $10^{-6}$  M,  $10^{-7}$  M,  $10^{-8}$  M,  $10^{-9}$  M or  $10^{-10}$  M or less. In some embodiment examples of the present invention disclosure, the term "target" refers to specific binding.

As used herein, the term " $K_D$ " refers to a dissociation equilibrium constant for a specific antibody-antigen interaction, which is used to describe the binding affinity between the antibody and the antigen. The smaller the equilibrium dissociation constant, the tighter the antibody-antigen binding, and the higher the affinity between the antibody and the antigen. Generally, an antibody binds to an antigen with a dissociation equilibrium constant ( $K_D$ ) of less than about  $10^{-5}$  M, such as less than about  $10^{-6}$  M,  $10^{-7}$  M,  $10^{-8}$  M,  $10^{-9}$  M or  $10^{-10}$  M or less, for example, as measured by a BIACORE surface plasmon resonance (SPR) instrument or a Fortebio Octet system.

As used herein, the terms "monoclonal antibody" and "mAb" have the same meaning and can be used interchangeably; the terms "polyclonal antibody" and "pAb" have the same meaning and can be used interchangeably; the terms "polypeptide" and "protein" have the same meaning and can be used interchangeably. Besides, herein, amino acids are generally represented by single-letter and three-letter abbreviations known in the art. For example, alanine can be represented by A or Ala.

As used herein, the term "pharmaceutically acceptable carrier and/or excipient" refers to a carrier and/or excipient that is pharmacologically and/or physiologically compatible with the subject and the active ingredient, which is well known in the art (see, e.g., *Remington's Pharmaceutical Sciences*, edited by Gennaro AR, 19th ed., Pennsylvania, Mack Publishing Company, 1995), including but not limited to: pH regulators, surfactants, adjuvants, and ionic strength enhancers. For example, the pH regulators include, but are not limited to, phosphate buffer; the surfactants include, but are not limited to, cationic, anionic, or non-ionic surfactants, such as Tween-80; the ionic strength enhancers include, but are not limited to, sodium chloride.

As used herein, the term "adjuvant" refers to a non-specific immune enhancer, which can enhance the immune response of an organism to antigens or change the type of immune response when delivered into the organism together with the antigens or delivered into the organism in advance. There are various adjuvants, including, but not limited to, aluminum adjuvant (e.g., aluminum hydroxide), Freund's adjuvant (e.g., complete Freund's adjuvant and incomplete Freund's adjuvant), *Corynebacterium parvum*, lipopolysaccharide, cytokine, etc. The Freund's adjuvant is the most commonly used adjuvant in animal experiments. The aluminum hydroxide adjuvant is used more in clinical trials.

As used herein, the term "effective amount" refers to an amount sufficient to obtain or at least partially obtain desired effect. For example, a prophylactically effective amount (e.g., for a disease associated with binding of CTLA4 to B7 or CTLA4 overactivity, such as a

tumor) is an amount sufficient to prevent, stop, or delay the onset of the disease (e.g., a disease associated with binding of CTLA4 to B7 or CTLA4 overactivity, such as a tumor); a therapeutically effective amount is an amount sufficient to cure or at least partially stop a disease and its complications in a patient suffering from the disease. It is undoubtedly within the ability of those skilled in the art to determine such an effective amount. For example, the amount effective for therapeutic purpose will depend on the severity of the disease to be treated, the overall state of the patient's own immune system, the general condition of the patient such as age, weight and gender, the route of administration, and other treatments given concurrently, etc.

A "recurrent" cancer is one that regenerates at the original site or a distant site after response to a previous treatment (e.g., surgery). A "locally recurrent" cancer is one that occurs at the same site after treatment as the previously treated cancer.

A "metastatic" cancer refers to one that spreads from one part of the body (e.g., the lungs) to another.

As used herein, the term "completely eliminated" refers to the absence of binding signal or an extremely weak binding signal as detected by existing instrumentation (e.g., a Fortebio Octet system). In one **embodimentexample** of the present **inventiondisclosure**, the absence of binding signal or the extremely weak binding signal refers to a binding signal (i.e., response) below 0.1 nm.

In the present **inventiondisclosure**, the terms "first" (e.g., first protein functional region, first linker fragment or first product) and "second" (e.g., second protein functional region, second linker fragment or second product) are used for distinguishing or clarity in expression and do not carry typical sequential meanings, unless otherwise specified.

In the present **inventiondisclosure**, "about" or "approximately" refers to that the value or physical quantity defined fluctuates within a range of 10%, 20% or 30%, unless otherwise specified. For example, about 100 minutes or approximately 100 minutes may be 90 minutes to 110 minutes, 80 minutes to 120 minutes or 70 minutes to 130 minutes.

Advantages of the present **inventiondisclosure**:

The present **inventiondisclosure** achieves one or more of the following technical effects (1) to (3):

(1) The modification of the Fc fragment of the antibody of the present **inventiondisclosure** by the inventor completely eliminates the binding activity of BiAb004(hG1TM) to FcγRI, FcγRIIIa\_H131, FcγRIIIa\_V158 and/or FcγRIIIa\_F158, thereby eliminating the ADCC activity and/or ADCP activity.

(2) The modification of the Fc fragment of the antibody of the present **inventiondisclosure** by the inventor completely eliminates the binding activity to complement C1q, thereby eliminating the CDC activity.

(3) The antibody of the present **inventiondisclosure** has potential for use in preparing medicaments for preventing and treating tumors.

## CLAIMS

1. A bispecific antibody, comprising:

a first protein functional region targeting PD-1, and

a second protein functional region targeting CTLA4;

wherein the first protein functional region is an immunoglobulin, and the second protein functional region is a single chain antibody; or, the first protein functional region is a single chain antibody, and the second protein functional region is an immunoglobulin;

wherein,

for the immunoglobulin, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 27–29 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 30–32 respectively; for the single chain antibody, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 33–35 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 36–38 respectively;

or,

for the immunoglobulin, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 33–35 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 36–38 respectively; for the single chain antibody, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 27–29 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 30–32 respectively;

the immunoglobulin is of human IgG1 subtype;

wherein, according to the EU numbering system, the heavy chain constant region of the immunoglobulin has mutations at any 2 or 3 of positions 234, 235 and 237, and the affinity constant of the bispecific antibody to FcγRIIIa and/or C1q is reduced after the mutation as compared to that before the mutation; preferably, the affinity constant is measured by a Fortebio Octet system.

2. The bispecific antibody according to claim 1, wherein, according to the EU numbering system, the heavy chain constant region of the immunoglobulin has the following mutations:

L234A and L235A;

L234A and G237A;

L235A and G237A;

or

L234A, L235A and G237A.

3. A bispecific antibody, comprising:

a first protein functional region targeting PD-1, and

a second protein functional region targeting CTLA4;

wherein the first protein functional region is an immunoglobulin, and the second protein functional region is a single chain antibody; or, the first protein functional region is a single chain antibody, and the second protein functional region is an immunoglobulin;

wherein,

for the immunoglobulin, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 27–29 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 30–32 respectively; for the single chain antibody, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 33–35 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 36–38 respectively;

or,

for the immunoglobulin, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 33–35 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 36–38 respectively; for the single chain antibody, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 27–29 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 30–32 respectively;

the immunoglobulin is of human IgG1 subtype;

wherein, according to the EU numbering system, the heavy chain constant region of the immunoglobulin has the following mutations:

L234A and L235A;

L234A and G237A;

L235A and G237A; or

L234A, L235A and G237A.

4. The bispecific antibody according to any of claims 1–3, wherein, according to the EU

numbering system, the heavy chain constant region of the immunoglobulin has one or more mutations selected from:

N297A, D265A, D270A, P238D, L328E, E233D, H268D, P271G, A330R, C226S, C229S, E233P, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, N297Q, P238S, P238A, A327Q, A327G, P329A, K322A, T394D, G236R, G236A, L328R, A330S, P331S, H268A, E318A and K320A.

5. The bispecific antibody according to any of claims 1–4, wherein,

the amino acid sequence of the heavy chain variable region of the immunoglobulin is selected from SEQ ID NO: 14 and SEQ ID NO: 18; the amino acid sequence of the light chain variable region of the immunoglobulin is selected from SEQ ID NO: 16 and SEQ ID NO: 20; 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: 41 and SEQ ID NO: 43; 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, SEQ ID NO: 42 and SEQ ID NO: 44;

or,

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: 41 and SEQ ID NO: 43; the amino acid sequence of the light chain variable region of the immunoglobulin is selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 42 and SEQ ID NO: 44; the amino acid sequence of the heavy chain variable region of the single chain antibody is selected from SEQ ID NO: 14 and SEQ ID NO: 18; the amino acid sequence of the light chain variable region of the single chain antibody is selected from SEQ ID NO: 16 and SEQ ID NO: 20.

6. The bispecific antibody according to any of claims 1–5, wherein the bispecific antibody is selected from any one of the following (1)–(20):

(1)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 16; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 2, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 4;

(2)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 16; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 6, and the amino acid

sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 8;

(3)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 16; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 12;

(4)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 18, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 20; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 2, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 4;

(5)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 18, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 20; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 6, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 8;

(6)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 18, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 20; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 12;

(7)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 2, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 4; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID

NO: 16;

(8)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 2, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 4; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 18, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 20;

(9)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 6, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 8; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 16;

(10)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 6, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 8; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 18, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 20;

(11)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 12; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 16;

(12)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 12; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 18, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 20;

(13)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 16; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 41, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 42;

(14)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 16; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 43, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 44;

(15)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 18, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 20; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 41, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 42;

(16)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 18, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 20; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 43, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 44;

(17)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 41, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 42; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 16;

(18)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 43, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 44; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 14, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 16;

(19)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 41, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 42; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 18, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 20;

and,

(20)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 43, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 44; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 18, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 20.

7. The bispecific antibody according to any of claims 1–6, wherein,

the amino acid sequence of the heavy chain of the immunoglobulin is set forth in SEQ ID NO: 40, and the amino acid sequence of the light chain of the immunoglobulin is set forth in SEQ ID NO: 24.

8. The bispecific antibody according to any of claims 1–7, wherein the immunoglobulin or the antigen-binding fragment thereof binds to FcγRIIIa\_F158, FcγRI, FcγRIIa\_H131, FcγRIIIa\_V158 and/or FcγRIIb with an affinity constant greater than about  $10^{-7}$  M, for example, greater than about  $10^{-6}$  M,  $10^{-5}$  M,  $10^{-4}$  M, or  $10^{-3}$  M or greater; preferably, the affinity constant is measured by a Fortebio Octet system;

preferably, the immunoglobulin or the antigen binding fragment thereof has no binding signal or a binding signal of less than 0.1 nm to FcγRIIIa\_F158, FcγRI, FcγRIIa\_H131, FcγRIIIa\_V158 and/or FcγRIIb; preferably, the binding signal refers to a response measured by a Fortebio Octet system.

9. The bispecific antibody according to any of claims 1–8, wherein the immunoglobulin or the antigen-binding fragment thereof binds to C1q with an affinity constant greater than about  $10^{-9}$  M, for example, greater than about  $10^{-8}$  M,  $10^{-7}$  M,  $10^{-6}$  M, or  $10^{-5}$  M or greater; preferably, the affinity constant is measured by a Fortebio Octet system;

preferably, the immunoglobulin or the antigen binding fragment thereof has no binding signal or a binding signal of less than 0.1 nm to C1q; preferably, the binding signal refers to a response measured by a Fortebio Octet system.

10. The bispecific antibody of any of claims 1–9, wherein the first protein functional region is linked to the second protein functional region either directly or via a linker fragment; and/or the heavy chain variable region of the single chain antibody is linked to the light chain variable region of the single chain antibody either directly or via a linker fragment; optionally wherein the linker fragment is (GGGS) $n$ ,  $n$  being a positive integer; preferably,  $n$  is 1, 2, 3, 4, 5 or 6.

~~11. The bispecific antibody of claim 10, wherein the linker fragment is (GGGS) $n$ ,  $n$  being a positive integer; preferably,  $n$  is 1, 2, 3, 4, 5 or 6.~~

~~12~~11. The bispecific antibody according to any of claims 1–~~11~~10, wherein the numbers of the first protein functional region and second protein functional region are each independently 1, 2 or more; optionally wherein the single chain antibody is linked to the C terminus of the heavy chain of the immunoglobulin.

~~13. The bispecific antibody according to any of claims 1–12, wherein the single chain antibody is linked to the C terminus of the heavy chain of the immunoglobulin.~~

~~14~~12. A bispecific antibody, comprising:

a first protein functional region targeting PD-1, and

a second protein functional region targeting CTLA4;

the number of the first protein functional region is 1, and the number of the second protein functional region is 2;

wherein the first protein functional region is an immunoglobulin, and the second protein functional region is a single chain antibody;

the amino acid sequence of the heavy chain of the immunoglobulin is set forth in SEQ ID NO: 40, and the amino acid sequence of the light chain of the immunoglobulin is set forth in SEQ ID NO: 24;

the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 43, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 44;

the single chain antibody is linked to the C terminus of the heavy chain of the immunoglobulin;

the first protein functional region is linked to the second protein functional region via a first linker fragment; and the heavy chain variable region of the single chain antibody is linked to the light chain variable region of the single chain antibody via a second linker fragment; the first linker fragment and the second linker fragment are the same or different;

preferably, the amino acid sequences of the first linker fragment and second linker fragment are independently selected from SEQ ID NO: 25 and SEQ ID NO: 26;

preferably, the amino acid sequences of the first linker fragment and second linker fragments are set forth in SEQ ID NO: 26.

~~15~~13. An isolated nucleic acid molecule, encoding the bispecific antibody according to any of claims 1–~~14~~12.

~~16~~14. A vector, comprising the isolated nucleic acid molecule according to claim ~~15~~13.

~~17~~15. A host cell, comprising the isolated nucleic acid molecule according to claim ~~15~~13 or the vector according to claim ~~16~~14.

~~18~~16. A conjugate comprising an antibody or antigen-binding fragment thereof, and a conjugated moiety, wherein the immunoglobulin is the bispecific antibody according to any of claims 1–~~14~~12, and the conjugated moiety is a detectable label; preferably, the conjugated moiety is a radioisotope, a fluorescent substance, a luminescent substance, a colored substance, or an enzyme.

~~19~~17. A kit, comprising the bispecific antibody according to any of claims 1–~~14~~12 or the conjugate according to claim ~~18~~16;

wherein preferably, the kit further comprises a second antibody capable of specifically recognizing the immunoglobulin or the antigen binding fragment thereof; optionally, the second antibody further comprises a detectable label, for example, a radioisotope, a fluorescent substance, a luminescent substance, a colored substance, or an enzyme.

~~20~~18. Use of the bispecific antibody according to any of claims 1–~~14~~12 or the conjugate according to claim ~~18~~16 in preparing a kit for detecting the presence or level of PD-1 and/or CTLA4 in a sample.

~~21~~19. A pharmaceutical composition, comprising the bispecific antibody according to any of claims 1–~~14~~12 or the conjugate according to claim ~~18~~16, wherein, optionally, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier and/or excipient.

~~22~~20. The pharmaceutical composition according to claim ~~21~~19, further comprising one or more anti-tumor chemotherapeutics;

preferably, the anti-tumor chemotherapeutic is a tyrosine kinase inhibitor; more preferably, the anti-tumor chemotherapeutic is anlotinib or a pharmaceutically acceptable salt thereof (e.g., hydrochloride salt), or lenvatinib or a pharmaceutically acceptable salt thereof (e.g., mesylate

salt); optionally wherein the unit dose of the pharmaceutical composition is 100–1000 mg, 200–800 mg, 200–500 mg, 300–600 mg, 400–500 mg, or 450 mg, based on the mass of the bispecific antibody.

~~23. The pharmaceutical composition according to claim 21 or 22, wherein the unit dose of the pharmaceutical composition is 100–1000 mg, 200–800 mg, 200–500 mg, 300–600 mg, 400–500 mg, or 450 mg, based on the mass of the bispecific antibody.~~

~~24~~21. A combination product comprising a first product and a second product in separate packages,

wherein,

the first product comprises the bispecific antibody according to any of claims 1–~~14~~12, the conjugate according to claim ~~18~~16, or the pharmaceutical composition according to any of claims ~~21~~19–23~~20~~;

the second product comprises one or more anti-tumor chemotherapeutics; preferably, the anti-tumor chemotherapeutic is a tyrosine kinase inhibitor; more preferably, the anti-tumor chemotherapeutic is anlotinib or a pharmaceutically acceptable salt thereof (e.g., hydrochloride salt), or lenvatinib or a pharmaceutically acceptable salt thereof (e.g., mesylate salt);

preferably, the first product and the second product further independently comprise one or more pharmaceutically acceptable excipients;

preferably, the combination product further comprises a package insert.

~~25~~22. The combination product according to claim 24, wherein the unit dose of the first product is 100–1000 mg, 200–800 mg, 200–500 mg, 300–600 mg, 400–500 mg, or 450 mg, based on the mass of the bispecific antibody; optionally wherein the unit dose of the second product is 0.1–100 mg, 0.5–50 mg, 1–20 mg, 2–15 mg, 4–12 mg, or 8–12 mg, based on the mass of the active ingredient.

~~26. The combination product according to claim 24 or 25, wherein the unit dose of the second product is 0.1–100 mg, 0.5–50 mg, 1–20 mg, 2–15 mg, 4–12 mg, or 8–12 mg, based on the mass of the active ingredient.~~

~~27~~23. Use of the bispecific antibody according to any of claims 1–~~14~~12 or the conjugate according to claim ~~18~~16 in preparing a medicament for treating and/or preventing a tumor or anemia, or in preparing a medicament for diagnosing a tumor or anemia;

preferably, the tumor is selected from one or more of melanoma, renal cancer, prostate cancer, bladder cancer, colon cancer, rectal cancer, gastric cancer, liver cancer, lung cancer, ovarian cancer, leukemia, breast cancer, mesothelioma, cervical cancer, endometrial cancer, lymphoma and nasopharyngeal cancer;

preferably, the lung cancer is selected from one or more of non-small cell lung cancer, small cell

lung cancer and squamous cell lung cancer;

preferably, the gastric cancer is gastric adenocarcinoma or gastroesophageal junction adenocarcinoma; preferably, the tumor is a solid tumor of MSI-H/dMMR phenotype; preferably, the tumor is selected from one or more of the following tumors of MSI-H/dMMR phenotype:

colon cancer, rectal cancer, endometrial cancer, gastric cancer, mesothelioma, sarcoma, adrenocortical carcinoma, malignant melanoma and ovarian germ cell neoplasm.

~~28~~24. Use of the bispecific antibody according to any of claims 1–~~14~~12 or the conjugate according to claim ~~18~~16 in preparing:

a medicament for blocking the binding of PD-1 to PD-L1,

a medicament for down-regulating the activity or level of PD-1,

a medicament for relieving the immunosuppression of PD-1 in an organism, or

a medicament for elevating IFN- $\gamma$  and/or IL-2 expression in T lymphocytes;

and/or

a medicament for blocking the binding of CTLA4 to B7,

a medicament for down-regulating the activity or level of CTLA4,

a medicament for relieving the immunosuppression of CTLA4 in an organism, or

a medicament for elevating IL-2 expression in T lymphocytes.

~~29. The bispecific antibody according to any of claims 1–14, the conjugate according to claim 18, the pharmaceutical composition according to any of claims 21–23 or the combination product according to any of claims 24–26 for use in treating and/or preventing a tumor or anemia, or for use in diagnosing a tumor or anemia;~~

~~preferably, the tumor is selected from one or more of melanoma, renal cancer, prostate cancer, bladder cancer, colon cancer, rectal cancer, gastric cancer, liver cancer, lung cancer, ovarian cancer, leukemia, breast cancer, mesothelioma, cervical cancer, endometrial cancer, lymphoma and nasopharyngeal cancer;~~

~~preferably, the lung cancer is selected from one or more of non-small cell lung cancer, small cell lung cancer and squamous cell lung cancer;~~

~~preferably, the gastric cancer is gastric adenocarcinoma or gastroesophageal junction adenocarcinoma;~~

~~preferably, the tumor is a solid tumor of MSI-H/dMMR phenotype; preferably, the tumor is selected from one or more of the following tumors of MSI-H/dMMR phenotype:~~

~~colon cancer, rectal cancer, endometrial cancer, gastric cancer, mesothelioma, sarcoma,~~

~~adrenocortical carcinoma, malignant melanoma and ovarian germ cell neoplasm.~~

~~30. A method for preventing and/or treating a tumor, comprising: administering to a subject in need an effective amount of the bispecific antibody according to any of claims 1-14, the conjugate according to claim 18, the pharmaceutical composition according to any of claims 21-23 or the combination product according to any one of claims 24-26;~~

~~preferably, the tumor is selected from one or more of melanoma, renal cancer, prostate cancer, bladder cancer, colon cancer, rectal cancer, gastric cancer, liver cancer, lung cancer, ovarian cancer, leukemia, breast cancer, mesothelioma, cervical cancer, endometrial cancer, lymphoma and nasopharyngeal cancer;~~

~~preferably, the lung cancer is selected from one or more of non-small cell lung cancer, small cell lung cancer and squamous cell lung cancer;~~

~~preferably, the gastric cancer is gastric adenocarcinoma or gastroesophageal junction adenocarcinoma;~~

~~preferably, the tumor is a solid tumor of MSI-H/dMMR phenotype; preferably, the tumor is selected from one or more of the following tumors of MSI-H/dMMR phenotype:~~

~~colon cancer, rectal cancer, endometrial cancer, gastric cancer, mesothelioma, sarcoma, adrenocortical carcinoma, malignant melanoma and ovarian germ cell neoplasm.~~

~~31. The method according to claim 30, wherein the administration is before or after a surgical treatment and/or before or after a radiotherapy.~~

~~32. The method according to claim 30 or 31, wherein the unit dose of the bispecific antibody is 0.1-100 mg per kg body weight, preferably 1-10 mg per kg body weight; alternatively, the unit dose of the bispecific antibody is 10-1000 mg, preferably 50-500 mg in each subject;~~

~~preferably, the dose is given once every 3 days, 4 days, 5 days, 6 days, 10 days, 1 week, 2 weeks or 3 weeks;~~

~~preferably, the route of administration is intravenous drip infusion or intravenous injection.~~

~~33. The method according to any of claims 30-32, wherein the administration of the bispecific antibody is performed in cycles of 2 or 3 weeks, and preferably, the bispecific antibody is administered intravenously on the first day of each cycle; preferably, the bispecific antibody is administered once every two or three weeks.~~