

CLAIMS

1. A bispecific antibody, comprising:

a first protein functional region targeting PD-1, and

a second protein functional region targeting VEGFA;

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: 28–30 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 31–33 respectively; for the single chain antibody, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 34–36 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 37–39 respectively;

or,

for the immunoglobulin, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 34–36 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 37–39 respectively; for the single chain antibody, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 28–30 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID

**NOs: 31–33 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; or

L234A and G237A; or

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 VEGFA;

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: 28–

30 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 31–33 respectively; for the single chain antibody, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 34–36 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 37–39 respectively;

or,

for the immunoglobulin, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 34–36 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 37–39 respectively; for the single chain antibody, the heavy chain variable region comprises HCDR1–HCDR3 of amino acid sequences set forth in SEQ ID NOs: 28–30 respectively, and the light chain variable region comprises LCDR1–LCDR3 of amino acid sequences set forth in SEQ ID NOs: 31–33 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; or

L234A and G237A; or

L235A and G237A; or

L234A, L235A and G237A.

4. The bispecific antibody according to any one 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 one of claims 1–4, wherein, the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 3; the amino acid sequence of the heavy chain variable region of the single chain antibody is selected from SEQ ID NO: 5 and SEQ ID NO: 9, and the amino acid sequence of the light chain variable region of the single chain antibody is selected from SEQ ID NO: 7, SEQ ID NO: 11 and SEQ ID NO: 17;

or,

the amino acid sequence of the heavy chain variable region of the immunoglobulin is selected from SEQ ID NO: 5 and SEQ ID NO: 9, and the amino acid sequence of the light chain variable region of the immunoglobulin is selected from SEQ ID NO: 7, SEQ ID NO: 11 and SEQ ID NO: 17; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 3.

6. The bispecific antibody according to any one of claims 1–5, wherein the bispecific antibody is selected from any one of the following (1)–(12):

(1)

the amino acid sequence of the heavy chain variable region of the

immunoglobulin is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 3; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 5, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 7;

(2)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 3; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 5, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 11;

(3)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 3; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 5, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 17;

(4)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 3; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 9,

and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 7;

(5)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 3; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 9, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 11;

(6)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 3; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 9, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 17;

(7)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 5, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 7; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 3;

(8)

the amino acid sequence of the heavy chain variable region of the

immunoglobulin is set forth in SEQ ID NO: 5, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 11; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 3;

(9)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 5, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 17; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 3;

(10)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 9, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 7; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 3;

(11)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 9, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 11; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 1,

and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 3; and

(12)

the amino acid sequence of the heavy chain variable region of the immunoglobulin is set forth in SEQ ID NO: 9, and the amino acid sequence of the light chain variable region of the immunoglobulin is set forth in SEQ ID NO: 17; the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 1, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 3.

7. The bispecific antibody according to any one of claims 1–6, wherein, the amino acid sequence of the heavy chain of the immunoglobulin is set forth in SEQ ID NO: 24, and the amino acid sequence of the light chain of the immunoglobulin is set forth in SEQ ID NO: 26.

8. The bispecific antibody according to any one of claims 1-7, wherein the immunoglobulin or an antigen-binding fragment thereof binds to FcγRI with an affinity constant of less than about 10^{-6} M, such as less than about 10^{-7} M, 10^{-8} M or 10^{-9} M or less; preferably, the affinity constant is measured by a Fortebio Octet system.

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

10. The bispecific antibody according to any one 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.

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

12. The bispecific antibody according to any one of claims 1–11, wherein the numbers of the first protein functional region and second protein functional region are each independently 1, 2 or more.

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

14. A bispecific antibody, comprising:

a first protein functional region targeting PD-1, and

a second protein functional region targeting VEGFA;

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

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: 24, and the amino acid sequence of the light chain

**of the immunoglobulin is set forth in SEQ ID NO: 26;
the amino acid sequence of the heavy chain variable region of the single chain antibody is set forth in SEQ ID NO: 9, and the amino acid sequence of the light chain variable region of the single chain antibody is set forth in SEQ ID NO: 17;
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: 18 and SEQ ID NO: 19;
preferably, the amino acid sequences of the first linker fragment and second linker fragments are set forth in SEQ ID NO: 18.**

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

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

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

18. A conjugate, comprising an antibody or an antigen-binding fragment

thereof and a conjugated moiety, wherein the immunoglobulin is the bispecific antibody according to any one of claims 1–14, 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. A kit, comprising the bispecific antibody according to any one of claims 1–14 or the conjugate according to claim 18; 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. Use of the bispecific antibody according to any one of claims 1–14 or the conjugate according to claim 18 in the preparation of a kit for detecting the presence or level of PD-1 and/or VEGFA in a sample.

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

22. Use of the bispecific antibody according to any one of claims 1–14 or the conjugate according to claim 18 in the preparation of a medicament for treating and/or preventing a malignant tumor; preferably, the malignant tumor is selected from colon cancer, rectal cancer, lung cancer, liver cancer, ovarian cancer, skin cancer, glioma, melanoma, lymphoma,

renal tumor, prostate cancer, bladder cancer, gastrointestinal cancer, breast cancer, brain cancer, cervical cancer, esophageal cancer, microsatellite instability-high (MSI-H) and deficient mismatch repair (dMMR) cancer, urothelial cancer, mesothelioma, endometrial cancer, gastric adenocarcinoma, gastroesophageal junction adenocarcinoma and leukemia;

preferably, the lung cancer is non-small cell lung cancer or small cell lung cancer; preferably, the non-small cell lung cancer is EGFR and/or ALK sensitive mutant non-small cell lung cancer;

preferably, the liver cancer is hepatocellular carcinoma;

preferably, the renal tumor is renal cell carcinoma;

preferably, the breast cancer is triple negative breast cancer;

preferably, the urothelial cancer is bladder cancer.

~~23. A method for treating and/or preventing a malignant tumor, comprising a step of administering to a subject in need thereof an effective amount of the bispecific antibody according to any one of claims 1-14 or the conjugate according to claim 18; preferably, the malignant tumor is selected from colon cancer, rectal cancer, lung cancer, liver cancer, ovarian cancer, skin cancer, glioma, melanoma, lymphoma, renal tumor, prostate cancer, bladder cancer, gastrointestinal cancer, breast cancer, brain cancer, cervical cancer, esophageal cancer, microsatellite instability-high (MSI-H) and deficient mismatch repair (dMMR) cancer, urothelial cancer, mesothelioma, endometrial cancer, gastric adenocarcinoma, gastroesophageal junction adenocarcinoma and leukemia;~~

~~preferably, the lung cancer is non-small cell lung cancer or small cell lung cancer; preferably, the non-small cell lung cancer is EGFR and/or ALK~~

~~sensitive mutant non-small cell lung cancer;~~
~~preferably, the liver cancer is hepatocellular carcinoma;~~
~~preferably, the renal tumor is renal cell carcinoma;~~
~~preferably, the breast cancer is triple negative breast cancer;~~
~~preferably, the urothelial cancer is bladder cancer.~~

2423. The bispecific antibody according to any one of claims 1–14 for use in the treatment and/or prevention of a malignant tumor; preferably, the malignant tumor is selected from colon cancer, rectal cancer, lung cancer, liver cancer, ovarian cancer, skin cancer, glioma, melanoma, lymphoma, renal tumor, prostate cancer, bladder cancer, gastrointestinal cancer, breast cancer, brain cancer, cervical cancer, esophageal cancer, microsatellite instability-high (MSI-H) and deficient mismatch repair (dMMR) cancer, urothelial cancer, mesothelioma, endometrial cancer, gastric adenocarcinoma, gastroesophageal junction adenocarcinoma and leukemia;

preferably, the lung cancer is non-small cell lung cancer or small cell lung cancer; preferably, the non-small cell lung cancer is EGFR and/or ALK sensitive mutant non-small cell lung cancer;

preferably, the liver cancer is hepatocellular carcinoma;

preferably, the renal tumor is renal cell carcinoma;

preferably, the breast cancer is triple negative breast cancer;

preferably, the urothelial cancer is bladder cancer.

2524. The conjugate according to claim 18 for use in the treatment and/or prevention of a malignant tumor; preferably, the malignant tumor is selected from colon cancer, rectal cancer, lung cancer, liver cancer, ovarian cancer, skin cancer, glioma, melanoma, lymphoma, renal tumor, prostate

cancer, bladder cancer, gastrointestinal cancer, breast cancer, brain cancer, cervical cancer, esophageal cancer, microsatellite instability-high (MSI-H) and deficient mismatch repair (dMMR) cancer, urothelial cancer, mesothelioma, endometrial cancer, gastric adenocarcinoma, gastroesophageal junction adenocarcinoma and leukemia;
preferably, the lung cancer is non-small cell lung cancer or small cell lung cancer; preferably, the non-small cell lung cancer is EGFR and/or ALK sensitive mutant non-small cell lung cancer;
preferably, the liver cancer is hepatocellular carcinoma;
preferably, the renal tumor is renal cell carcinoma;
preferably, the breast cancer is triple negative breast cancer;
preferably, the urothelial cancer is bladder cancer.