

## **CLAIMS**

1. A bispecific antibody, comprising:

a first protein functional region targeting VEGFA, and

a second protein functional region targeting PD-1;

wherein:

the first protein functional region is an anti-VEGFA antibody or an antigen-binding fragment thereof, a heavy chain variable region of the anti-VEGFA antibody comprising HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NOs: 15-17 respectively, and a light chain variable region of the anti-VEGFA antibody comprising LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NOs: 18-20 respectively; and

the second protein functional region is an anti-PD-1 antibody or an antigen-binding fragment thereof, a heavy chain variable region of the anti-PD-1 antibody comprising HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NOs: 21-23 respectively, and a light chain variable region of the anti-PD-1 antibody comprising LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NOs: 24-26 respectively.

2. The bispecific antibody according to claim 1, wherein,

the anti-VEGFA antibody or the antigen-binding fragment thereof is selected from Fab, Fab', F(ab')<sub>2</sub>, Fd, Fv, dAb, a complementarity determining region fragment, a single chain antibody, a humanized antibody, a chimeric antibody, and a diabody;

and/or,

the anti-PD-1 antibody or the antigen-binding fragment thereof is selected from Fab, Fab', F(ab')<sub>2</sub>, Fd, Fv, dAb, a complementarity determining region fragment, a single chain antibody, a humanized antibody, a chimeric antibody, and a diabody.

3. The bispecific antibody according to claim 1, wherein,

the first protein functional region is an immunoglobulin, a heavy chain variable region of the immunoglobulin comprising HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NOs: 15-17 respectively, and a light chain variable region of the immunoglobulin comprising LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NOs: 18-20 respectively; and the second protein functional region is a single chain antibody, a heavy chain variable region of the single chain antibody comprising HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NOs: 21-23 respectively, and a light chain variable region of the single chain antibody comprising LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NOs: 24-26 respectively;

or,

~~the first protein functional region is a single chain antibody, a heavy chain variable region of the single chain antibody comprising HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NOs: 21-23 respectively, and a light chain variable region of the single chain antibody comprising LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NOs: 24-26 respectively; and the second protein functional region is an immunoglobulin, a heavy chain variable region of the immunoglobulin comprising HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NOs: 15-17 respectively, and a light chain variable region of the immunoglobulin comprising LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NOs: 18-20 respectively.~~

4. The bispecific antibody according to claim 1, wherein,

the first protein functional region is a single chain antibody, a heavy chain variable region of the single chain antibody comprising HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NOs: 15-17 respectively, and a light chain variable region of the single chain antibody comprising LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NOs: 18-20 respectively; and the second

protein functional region is an immunoglobulin, a heavy chain variable region of the immunoglobulin comprising HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NOs: 21-23 respectively, and a light chain variable region of the immunoglobulin comprising LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NOs: 24-26 respectively.

45. The bispecific antibody according to claim 3, wherein,  
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; and 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;

or,

~~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; and 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.~~

6. The bispecific antibody according to claim 4, wherein,  
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; and 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.

57. The bispecific antibody according to ~~claim 3 or 4~~anyone of claims 3-6, wherein the immunoglobulin is an IgG, IgA, IgD, IgE, or IgM; preferably, the

immunoglobulin is an IgG.

~~68~~68. The bispecific antibody according to ~~claim 3 or 4~~anyone of claims 3-6, wherein two single chain antibodies are present, and one terminus of each single chain antibody is linked to the C-terminus or the N-terminus of one of the two heavy chains of the immunoglobulin.

~~79~~79. The bispecific antibody according to ~~claim 3 or 4~~anyone of claims 3-6, wherein,

the immunoglobulin comprises a non-CDR region derived from a species other than murine, such as from a human antibody.

~~810~~810. The bispecific antibody according to ~~claim 3 or 4~~anyone of claims 3-6, wherein,

the immunoglobulin comprises constant regions derived from a human antibody;

preferably, the constant regions of the immunoglobulin are selected from constant regions of human IgG1, IgG2, IgG3, and IgG4.

~~911~~911. The bispecific antibody according to ~~claim 3 or 4~~anyone of claims 3-6, wherein,

the heavy chain constant region of the immunoglobulin is human Ig gamma-1 chain C region or human Ig gamma-4 chain C region, and its light chain constant region is human Ig kappa chain C region.

~~1012~~1012. The bispecific antibody according to claim 1 or 2, wherein the first and second protein functional regions are linked directly or via a linker fragment;

preferably, the linker fragment is (GGGGS)<sub>m</sub>, wherein m is a positive integer such as 1, 2, 3, 4, 5, or 6.

~~1113~~1113. The bispecific antibody according to claim 1 or 2, wherein the numbers of the first and second protein functional regions are each independently 1, 2 or more.

~~1214~~1214. The bispecific antibody according to claim 1 or 2, wherein,

the bispecific antibody binds to the VEGFA protein with an EC<sub>50</sub> of less than 1 nM, less than 0.5 nM, less than 0.2 nM, less than 0.15 nM, or less than 0.14 nM; preferably, the EC<sub>50</sub> is detected by indirect ELISA;

and/or,

the bispecific antibody binds to the PD-1 protein with an EC<sub>50</sub> of less than 1 nM, less than 0.5 nM, less than 0.2 nM, less than 0.17 nM, less than 0.16 nM, or less than 0.15 nM; preferably, the EC<sub>50</sub> is detected by indirect ELISA.

[1315](#). An isolated nucleic acid molecule, encoding the bispecific antibody according to any one of claims 1-[1214](#).

[1416](#). A vector, comprising the isolated nucleic acid molecule according to claim [1315](#).

[1517](#). A host cell, comprising the isolated nucleic acid molecule according to claim [1315](#) or the vector according to claim [1416](#).

[1618](#). A method for preparing the bispecific antibody according to any one of claims 1-[1214](#), comprising: culturing the host cell according to claim [1517](#) in a suitable condition, and isolating the bispecific antibody from the cell cultures.

[1719](#). A conjugate, comprising a bispecific antibody and a conjugated moiety, wherein the bispecific antibody is the bispecific antibody according to any one of claims 1-[1214](#), 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.

[1820](#). A kit, comprising the bispecific antibody according to any one of claims 1-[1214](#) or the conjugate according to claim [1719](#);

wherein preferably, the kit further comprises a second antibody capable of specifically binding to the bispecific antibody; optionally, the second antibody further comprises a detectable label, such as a radioisotope, a fluorescent substance, a luminescent substance, a colored substance, or an enzyme.

[1921](#). Use of the bispecific antibody according to any one of claims 1-[1214](#) in

preparing a kit for detecting the presence or level of VEGFA and/or PD-1 in a sample.

[2022](#). A pharmaceutical composition, comprising the bispecific antibody according to any one of claims 1-[12-14](#) or the conjugate according to claim [1719](#), and optionally, a pharmaceutically acceptable excipient.

[2123](#). Use of the bispecific antibody according to any one of claims 1-[12-14](#) or the conjugate according to claim [17-19](#) in preparing a medicament for ~~preventing and/or~~ treating a malignant tumor, wherein preferably, the malignant tumor is selected from colon cancer, rectal cancer, lung cancer such as non-small cell lung cancer, liver cancer, ovarian cancer, skin cancer, glioma, melanoma, renal tumor, prostate cancer, bladder cancer, gastrointestinal cancer, breast cancer, brain cancer and leukemia.

[2224](#). Use of the bispecific antibody according to any one of claims 1-[12-14](#) or the conjugate according to claim [17-19](#) in preparing:

(1)

a medicament or an agent for detecting the level of VEGFA in a sample,  
a medicament or an agent for blocking the binding of VEGFA to VEGFR2,  
a medicament or an agent for down-regulating the activity or level of VEGFA,  
a medicament or an agent for relieving the stimulation of VEGFA on vascular endothelial cell proliferation,

a medicament or an agent for inhibiting vascular endothelial cell proliferation,  
or

a medicament or an agent for blocking tumor angiogenesis;  
and/or

(2)

a medicament or an agent for blocking the binding of PD-1 to PD-L1,  
a medicament or an agent for down-regulating the activity or level of PD-1,  
a medicament or an agent for relieving the immunosuppression of PD-1 in an

organism,

a medicament or an agent for promoting IFN- $\gamma$  secretion in T lymphocytes, or  
a medicament or an agent for promoting IL-2 secretion in T lymphocytes.

~~23~~25. The bispecific antibody according to any one of claims 1-~~12~~14 or the conjugate according to claim ~~17~~19 for use in ~~preventing and/or~~ treating a malignant tumor, wherein ~~preferably~~, the malignant tumor is selected from colon cancer, rectal cancer, lung cancer such as non-small cell lung cancer, liver cancer, ovarian cancer, skin cancer, glioma, melanoma, renal tumor, prostate cancer, bladder cancer, gastrointestinal cancer, breast cancer, brain cancer and leukemia.

~~24~~26. The bispecific antibody according to any one of claims 1-~~12~~14 or the conjugate according to claim ~~17~~19 for use in:

(1)

detecting the level of VEGFA in a sample,  
blocking the binding of VEGFA to VEGFR2,  
down-regulating the activity or level of VEGFA,  
relieving the stimulation of VEGFA on vascular endothelial cell proliferation,  
inhibiting vascular endothelial cell proliferation, or  
blocking tumor angiogenesis;  
and/or

(2)

blocking the binding of PD-1 to PD-L1,  
down-regulating the activity or level of PD-1,  
relieving the immunosuppression of PD-1 in an organism,  
promoting IFN- $\gamma$  secretion in T lymphocytes, or  
promoting IL-2 secretion in T lymphocytes.