

(9) HCDR1 shown in SEQ ID NO: 55, HCDR2 shown in SEQ ID NO: 56 and HCDR3 shown in SEQ ID NO: 57.

2. The anti-ANG2 heavy-chain single-domain antibody according to item 1, wherein ANG2 is selected from human ANG2, rabbit ANG2 or monkey ANG2.

- 5 3. The anti-ANG2 heavy-chain single-domain antibody according to item 1 or 2, comprising a heavy chain variable region, wherein the heavy chain variable region comprises or consists of a sequence shown in any one of SEQ ID NOs: 1 to 10 or a sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology with the sequence shown in any one of SEQ ID NOs: 1 to 10 and having ANG2 binding activity.
- 10 4. A recombinant protein, comprising the anti-ANG2 heavy-chain single-domain antibody according to any one of items 1 to 3, preferably, the recombinant protein further comprises a bioactive substance, for example, a toxin having enzymatic activity or an active fragment (for example, abrin, ricin A, pseudomonas exotoxin or diphtheria toxin) of the toxin, a tumor necrosis factor or an interferon (for example, IFN- γ), a biological response modifier (for
- 15 example, lymphokines IL-2, IL-2, IL2-6, IL-10 and GM-CSF) and/or an Fc fragment (for example, from an Fc region of IgG (for example, an IgG1, IgG2, IgG3 or IgG4 subtype), IgA1, IgA2, IgD, IgE or IgM, for example, from an Fc region of human, rabbit or monkey IgG, IgA1, IgD, IgE or IgM), preferably, the anti-ANG2 heavy-chain single-domain antibody and the Fc fragment are linked by a linker peptide, preferably, the linker peptide is GGGGS(SEQ ID NO:
- 20 30), GGGGSGGGGS (SEQ ID NO: 60) or GGGGSGGGGSGGGGS (SEQ ID NO: 47), preferably, the Fc fragment is a human IgG1 heavy chain constant region, more preferably an Fc fragment shown in GenBank No. AK303185.1 or SEQ ID NO: 29, more preferably, the recombinant protein comprises or consists of sequences shown in SEQ ID NOs: 11 to 20.
- 25 5. A multi-specific antibody, preferably a bispecific antibody, comprising the anti-ANG2 heavy-chain single-domain antibody according to any one of items 1 to 3, preferably, a structure of the bispecific antibody comprises a light chain and a heavy chain, wherein the light chain and the heavy chain are paired to form an interchain disulfide bond, and two heavy chains are paired to form an interchain disulfide bond, wherein the heavy chain is (VH)-(CH1)-(hinge region)-(Fc)-(linker peptide)-(VHH), and the light chain is (VL)-(light chain constant region).
- 30 6. The multi-specific antibody according to item 5, wherein VHH is the anti-ANG2 heavy-chain single-domain antibody according to any one of items 1 to 3, VH and VL are heavy chain and light chain variable regions of a second antibody, preferably, an antigen targeted by the second antibody is selected from an immune cell surface antigen, a tumor antigen, a virus, a bacterium, an endotoxin, a cytokine or a combination thereof; more preferably, the antigen targeted by the
- 35 second antibody is selected from PD-L1, PD-1, VEGFA, IL-10, IL-10R, BCMA, VEGF, TGF- β , CTLA-4, LAG-3, TIGIT, CEA, CD38, SLAMF7, B7-H3, Her2, EpCAM, CD19, CD20, CD30, CD33, CD47, CD52, CD133, EGFR, GD2, GD3, GM2, RANKL, CD3 and/or CD16a,

HCl stopping solution was added in 100 μ L/well. After the stopping solution was added, values at 450 nm were read with a microplate reader within 30 minutes. The results are shown in the following Table 3:

Table 3 OD values obtained after heavy-chain single-domain antibodies bind to hANG2 antigens

Clone No.	OD Value Obtained after Binding to hANG2	OD Value Obtained after Binding to BSA
B1-G8	2.021	0.065
B1-G3	1.572	0.067
B4-D3	1.679	0.059
B2-E2	1.962	0.070
B2-F10	2.061	0.063
B4-C11	1.58	0.071
B1-H9	1.495	0.058
B3-H7	1.82	0.056
B3-C11	1.63	0.071
B2-A6	2.39	0.049

Example 3 Preparation of plasmids of anti-ANG2 heavy-chain single-domain antibodies-Fc

Coding DNA sequences corresponding to monoclonal antibody heavy chain variable regions (SEQ ID NOs: 1 to 10) cloned through PCR were ligated to coding DNA corresponding to a human IgG1 heavy chain constant region (GenBank No. AK303185.1) to construct heavy-chain antibodies to obtain the expression plasmids of the anti-ANG2 heavy-chain single-domain antibodies-Fc, and vectors were generally pcDNA3.1(-) (purchased from Invitrogen) or other eukaryotic expression vectors. A variable region and a constant region of a heavy chain antibody protein sequence were linked by a linker peptide, and the linker peptide was GGGGS(SEQ ID NO: 30), GGGSGGGGS (SEQ ID NO: 60) or GGGSGGGGSGGGGS (SEQ ID NO: 47).

Table 4 Amino acid sequences of anti-ANG2 heavy-chain single-domain antibodies-Fc

Clone No.	Amino Acid Sequence of Antibody	SEQ ID NO
B1-G 8-Fc	QVQLVESGGGLVQTGDSLRLSCVAPGISFSNRGMAWYRQ APGKQRELVA/AIDSAGSQRYAGSVKDRFTISRDNANTVF LQMNSLKPEDTAVYYCNQPRFNLWGQGTQVTVSS GGGSGGGGSGGGGS DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVQLHQLDNLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK	11
B1-G 3-Fc	EVQVVESGGGLVQPGGSLRLSCVASMPGISFSNRGMAW YRQAPGKQRELVA/AIDSAGSTRYQDSVKGRFTISRDNAN	12

What is claimed is:

1. An anti-ANG2 heavy-chain single-domain antibody, comprising HCDR1, HCDR2 and HCDR3 contained in a heavy chain variable region shown in SEQ ID NO: 4 or variants of HCDR1, HCDR2 and HCDR3,

5 preferably, according to an IMGT numbering system, comprising HCDR1, HCDR2 and HCDR3 selected from a group consisting of:

HCDR1 shown in SEQ ID NO: 40, HCDR2 shown in SEQ ID NO: 41 and HCDR3 shown in SEQ ID NO: 42, or variants of HCDR1 shown in SEQ ID NO: 40, HCDR2 shown in SEQ ID NO: 41 and HCDR3 shown in SEQ ID NO: 42, and a combination thereof, wherein the variants
10 each have one or more (preferably one, two or three) conservative amino acid mutations (preferably a substitution, an insertion or a deletion) compared with HCDR1 shown in SEQ ID NO: 40, HCDR2 shown in SEQ ID NO: 41 and HCDR3 shown in SEQ ID NO: 42, respectively, and an amino acid sequence that has an affinity for binding to ANG2 is retained,

preferably, HCDR1 shown in SEQ ID NO: 40, HCDR2 shown in SEQ ID NO: 41 and HCDR3
15 shown in SEQ ID NO: 42, wherein amino acids at positions 5 to 8 and positions 10 and 11 in CDR1 shown in SEQ ID NO: 40, amino acids at positions 2 to 5 and position 7 in CDR2 shown in SEQ ID NO: 41 and amino acids at position 1 and positions 3 to 8 in CDR3 shown in SEQ ID NO: 42 are selected from an amino acid X, and the amino acid X is selected from a group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr,
20 Trp, Tyr and Val,

preferably, a variant of HCDR1 shown in SEQ ID NO: 40, wherein glycine G at position 1 may be substituted by an amino acid selected from a group consisting of: Ala, Val, Leu and Ile; phenylalanine F at position 2 may be substituted by Tyr; proline P at position 3 may be substituted by an amino acid selected from a group consisting of: Trp and His; Ser at position 4
25 may be substituted by Thr; Ser at position 9 may be substituted by Thr; and Gln at position 12 may be substituted by Asn;

a variant of HCDR2 shown in SEQ ID NO: 41, wherein Ile at position 1 may be substituted by an amino acid selected from a group consisting of: Ala, Val, leu and Gly; Leu at position 6 may be substituted by Ile; and Lys at position 8 may be substituted by Arg;

30 a variant of HCDR3 shown in SEQ ID NO: 42, wherein Val at position 2 may be substituted by an amino acid selected from a group consisting of: Ala, Gly, Leu and Ile; and Asp at position 9 may be substituted by Glu.

2. The anti-ANG2 heavy-chain single-domain antibody according to claim 1, wherein ANG2 is selected from human ANG2, rabbit ANG2 or monkey ANG2.

35 3. The anti-ANG2 heavy-chain single-domain antibody according to claim 1 or 2, comprising a

heavy chain variable region, wherein the heavy chain variable region comprises or consists of a sequence shown in SEQ ID NO: 4 or a sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology with the sequence shown in SEQ ID NO: 4 and having ANG2 binding activity.

5 4. A recombinant protein, comprising the anti-ANG2 heavy-chain single-domain antibody according to any one of claims 1 to 3, preferably, the recombinant protein further comprises a bioactive substance, for example, a toxin having enzymatic activity or an active fragment (for example, abrin, ricin A, pseudomonas exotoxin or diphtheria toxin) of the toxin, a tumor necrosis factor or an interferon (for example, IFN- γ), a biological response modifier (for example, lymphokines IL-2, IL-2, IL2-6, IL-10 and GM-CSF) and an Fc fragment (for example, from an Fc region of IgG (for example, an IgG1, IgG2, IgG3 or IgG4 subtype), IgA1, IgA2, IgD, IgE or IgM, for example, from an Fc region of human, rabbit or monkey IgG, IgA1, IgD, IgE or IgM), preferably, the anti-ANG2 heavy-chain single-domain antibody and the Fc fragment are linked by a linker peptide, preferably, the linker peptide is GGGGSGGGGSGGGGS (SEQ ID NO: 47), preferably, the Fc fragment is a human IgG1 heavy chain constant region, more preferably an Fc fragment shown in GenBank No. AK303185.1, more preferably, the recombinant protein comprises or consists of a sequence shown in SEQ ID NO: 14.

20 5. A multi-specific antibody, preferably a bispecific antibody, comprising the anti-ANG2 heavy-chain single-domain antibody according to any one of claims 1 to 3, preferably, a structure of the bispecific antibody comprises a light chain and a heavy chain, wherein the light chain and the heavy chain are paired to form an interchain disulfide bond, and two heavy chains are paired to form an interchain disulfide bond, wherein the heavy chain is (VH)-(CH1)-(hinge region)-(Fc)-(linker peptide)-(VHH), and the light chain is (VL)-(light chain constant region).

25 6. The multi-specific antibody according to claim 5, wherein VHH is the anti-ANG2 heavy-chain single-domain antibody according to any one of claims 1 to 3, VH and VL are heavy chain and light chain variable regions of a second antibody, preferably, an antigen targeted by the second antibody is selected from an immune cell surface antigen, a tumor antigen, a virus, a bacterium, an endotoxin, a cytokine or a combination thereof; more preferably, the antigen targeted by the second antibody is selected from PD-L1, PD-1, VEGFA, IL-10, IL-10R, BCMA, VEGF, TGF- β , CTLA-4, LAG-3, TIGIT, CEA, CD38, SLAMF7, 30 B7-H3, Her2, EpCAM, CD19, CD20, CD30, CD33, CD47, CD52, CD133, EGFR, GD2, GD3, GM2, RANKL, CD3 and/or CD16a, preferably, the antigen targeted by the second antibody is VEGF, more preferably, the second antibody is selected from an anti-VEGF antibody, the anti-VEGF antibody comprises HCDR1, HCDR2 and HCDR3 contained in a heavy chain variable region shown in SEQ ID NO: 24 (preferably, according to an IMGT numbering system, the anti-VEGF antibody comprises HCDR1 shown in SEQ ID NO: 50, HCDR2 shown in SEQ ID NO: 51 and HCDR3 shown in SEQ ID NO: 53) and LCDR1, LCDR2 and LCDR3 contained in a light chain variable region shown in SEQ ID NO: 27 (preferably, according to the IMGT

numbering system, the anti-VEGF antibody comprises LCDR1 shown in SEQ ID NO: 54, LCDR2 shown in SEQ ID NO: 58 and LCDR3 shown in SEQ ID NO: 59), more preferably, a heavy chain variable region of the anti-VEGF antibody comprises or consists of a sequence shown in SEQ ID NO: 24 or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology with the sequence shown in SEQ ID NO: 24, and a light chain variable region of the anti-VEGF antibody comprises or consists of a sequence shown in SEQ ID NO: 27 or a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology with the sequence shown in SEQ ID NO: 27.

7. The multi-specific antibody according to claim 5 or 6, wherein a sequence of CH1 is shown in SEQ ID NO: 25, a sequence of the hinge region is shown in SEQ ID NO: 26, a sequence of the Fc constant region is shown in SEQ ID NO: 29, a sequence of the linker peptide is shown in SEQ ID NO: 30 or 47, and a sequence of the light chain constant region is shown in SEQ ID NO: 28.

8. A polynucleotide, encoding the anti-ANG2 heavy-chain single-domain antibody according to any one of claims 1 to 3, the recombinant protein according to claim 4 or the multi-specific antibody according to any one of claims 5 to 7.

9. A vector, comprising the polynucleotide according to claim 8.

10. A host cell, comprising the vector according to claim 9.

11. A conjugate, comprising the anti-ANG2 heavy-chain single-domain antibody according to any one of claims 1 to 3, the recombinant protein according to claim 4, or the multi-specific antibody according to any one of claims 5 to 7, and a conjugated moiety, wherein the conjugated moiety is a purification tag (for example, a His tag), a detectable label, a drug, a toxin, a cytokine, an enzyme or a combination thereof; preferably, the conjugated moiety is a radioisotope, a fluorescent substance, a chemiluminescent substance, a colored substance, a chemotherapeutic agent, a biological toxin, polyethylene glycol or an enzyme.

12. A kit, comprising the anti-ANG2 heavy-chain single-domain antibody according to any one of claims 1 to 3, the recombinant protein according to claim 4, the multi-specific antibody according to any one of claims 5 to 7 or the conjugate according to claim 11; preferably, the kit further comprises an anti-ANG2 heavy-chain single-domain antibody, a recombinant protein or a multi-specific antibody specifically recognizing the anti-ANG2 heavy-chain single-domain antibody according to any one of claims 1 to 3, the recombinant protein according to claim 4, the multi-specific antibody according to any one of claims 5 to 7 or the conjugate according to claim 11; optionally, the anti-ANG2 heavy-chain single-domain antibody, the recombinant protein or the multi-specific antibody further comprises a detectable label, for example, a radioisotope, a fluorescent substance, a chemiluminescent substance, a colored substance or an enzyme; preferably, the kit is configured to detect the presence or a level of ANG2 in a sample;

or

the kit comprises: (1) the anti-ANG2 heavy-chain single-domain antibody according to any one of claims 1 to 3, the recombinant protein according to claim 4, the multi-specific antibody according to any one of claims 5 to 7 or the conjugate according to claim 11, and (2) an antibody or an antigen-binding fragment of the antibody that targets another antigen, and/or a cytotoxic agent, and/or a chemotherapeutic drug, and optionally, an instruction for use.

13. A pharmaceutical composition, comprising the anti-ANG2 heavy-chain single-domain antibody according to any one of claims 1 to 3, the recombinant protein according to claim 4, the multi-specific antibody according to any one of claims 5 to 7 or the conjugate according to claim 11, optionally, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier and/or an excipient, preferably, the pharmaceutical composition is in a form suitable for administration through subcutaneous injection, intradermal injection, intravenous injection, intramuscular injection or intralesional injection.

14. Use of the anti-ANG2 heavy-chain single-domain antibody according to any one of claims 1 to 3, the recombinant protein according to claim 4, the multi-specific antibody according to any one of claims 5 to 7 or the conjugate according to claim 11 in the manufacture of a medicament for inhibiting pro-angiogenesis activity of ANG2, preventing and/or treating diseases caused by or related to an angiogenic process such as fundus neovascular disease, rheumatoid arthritis and psoriasis, and/or a tumor, or for preparing drugs for inhibiting the pro-angiogenesis activity of ANG2, preventing and/or treating the diseases caused by or related to the angiogenic process such as fundus neovascular disease, rheumatoid arthritis and psoriasis, and/or the tumor.