

Claims

1. ~~An isolated antigen-binding protein having one or more of the following properties:~~

- ~~— (1) capable of binding VEGF protein with a K_D value of 1×10^{-7} M or lower; and~~
- ~~— (2) capable of blocking the binding of VEGF protein to VEGFR protein.~~

An antibody or antigen binding fragment capable of binding VEGF protein, which comprises HCDR1, HCDR2 and HCDR3, wherein the HCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 9, the HCDR2 comprises an amino acid sequence set forth in any one of SEQ ID NOs: 14, 13 or 12, and the HCDR3 comprises an amino acid sequence set forth in any one of SEQ ID NOs: 17-19.

2. ~~The isolated antigen-binding protein according to claim 1, wherein the VEGF protein includes VEGF165.~~

The antibody or antigen binding fragment according to claim 1, which comprises HCDR1, HCDR2 and HCDR3, and the HCDR1, HCDR2 and HCDR3 are selected from any one of the following groups of amino acid sequences:

- (1) HCDR1: SEQ ID NO: 9, HCDR2: SEQ ID NO: 14 and HCDR3: SEQ ID NO: 17;
- (2) HCDR1: SEQ ID NO: 9, HCDR2: SEQ ID NO: 12 and HCDR3: SEQ ID NO: 17;
- (3) HCDR1: SEQ ID NO: 9, HCDR2: SEQ ID NO: 13 and HCDR3: SEQ ID NO: 17;
- (4) HCDR1: SEQ ID NO: 9, HCDR2: SEQ ID NO: 13 and HCDR3: SEQ ID NO: 18;
- and
- (5) HCDR1: SEQ ID NO: 9, HCDR2: SEQ ID NO: 13 and HCDR3: SEQ ID NO: 19.

3. The antibody or antigen binding fragment according to claim 1 or 2, wherein the VEGF protein includes VEGF165.

3. ~~The isolated antigen-binding protein according to any one of claims 1-2, wherein the VEGFR protein includes VEGFR2.~~

4. ~~The isolated antigen-binding protein according to any one of claims 1-3, which includes an antibody or an antigen-binding fragment thereof.~~

5. ~~The isolated antigen-binding protein according to claim 4, wherein the antigen-binding fragment includes Fab, Fab', F(ab)₂, Fv fragment, F(ab')₂, scFv, di-scFv, VHH and/or dAb.~~

- ~~64. The antibody or antigen binding fragment according to any one of claims 1 to 3 isolated antigen-binding protein according to claim 4 or 5, wherein the antigen binding fragment is VHH.~~
- ~~75. The antibody or antigen binding fragment according to any one of claims 1 to 4, The isolated antigen-binding protein according to any one of claims 4-6, wherein the antibody is selected from the group consisting of: a monoclonal antibody, a chimeric antibody, a humanized antibody and a fully human antibody.~~
- ~~8. The isolated antigen-binding protein according to any one of claims 1-7, which is capable of competing with a reference antibody for binding to the VEGF protein, wherein the reference antibody comprises a heavy chain variable region VH, the VH of the reference antibody comprises HCDR1, HCDR2 and HCDR3, and the HCDR1 of the reference antibody comprises an amino acid sequence set forth in SEQ ID NO: 9, the HCDR2 of the reference antibody comprises an amino acid sequence set forth in SEQ ID NO: 23, and, the HCDR3 of the reference antibody comprises an amino acid sequence set forth in SEQ ID NO: 24.~~
- ~~9. The isolated antigen-binding protein according to claim 8, wherein the HCDR1 of the reference antibody comprises an amino acid sequence set forth in SEQ ID NO: 9.~~
- ~~10. The isolated antigen-binding protein according to any one of claims 8-9, wherein the HCDR2 of the reference antibody comprises an amino acid sequence set forth in any one of SEQ ID NOs: 12-14.~~
- ~~11. The isolated antigen-binding protein according to any one of claims 8-10, wherein the HCDR3 of the reference antibody comprises an amino acid sequence set forth in any one of SEQ ID NOs: 17-19.~~
- ~~12. The isolated antigen-binding protein according to any one of claims 1-11, which comprises at least one CDR in a VH, the VH comprising an amino acid sequence set forth in SEQ ID NO: 29.~~
- ~~13. The isolated antigen-binding protein according to any one of claims 1-12, which comprises at least one CDR in a VH, the VH comprising an amino acid sequence set forth in any one of SEQ ID NOs: 1-6.~~

- ~~14. The isolated antigen-binding protein according to any one of claims 1-13, which comprises HCDR3, and the HCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 24.~~
- ~~15. The isolated antigen-binding protein according to claim 14, wherein the HCDR3 comprises an amino acid sequence set forth in any one of SEQ ID NOs: 17-19.~~
- ~~16. The isolated antigen-binding protein according to any one of claims 1-15, which comprises HCDR2, and the HCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 23.~~
- ~~17. The isolated antigen-binding protein according to claim 16, wherein the HCDR2 comprises an amino acid sequence set forth in any one of SEQ ID NOs: 12-14.~~
- ~~18. The isolated antigen-binding protein according to any one of claims 1-17, which comprises HCDR1, and the HCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 9.~~
- ~~19. The isolated antigen-binding protein according to any one of claims 1-18, which comprises HCDR1, HCDR2 and HCDR3, wherein the HCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 9, the HCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 23, and the HCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 24.~~
- ~~20. The isolated antigen-binding protein according to any one of claims 1-19, which comprises HCDR1, HCDR2 and HCDR3, wherein the HCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 9, the HCDR2 comprises an amino acid sequence set forth in any one of SEQ ID NOs: 12-14, and the HCDR3 comprises an amino acid sequence set forth in any one of SEQ ID NOs: 17-19.~~
- ~~21. The isolated antigen-binding protein according to any one of claims 1-20, which comprises HCDR1, HCDR2 and HCDR3, and the HCDR1, HCDR2 and HCDR3 are selected from any one of the following groups of amino acid sequences:
(1) HCDR1: SEQ ID NO: 9, HCDR2: SEQ ID NO: 12 and HCDR3: SEQ ID NO: 17;
(2) HCDR1: SEQ ID NO: 9, HCDR2: SEQ ID NO: 13 and HCDR3: SEQ ID NO: 17;
(3) HCDR1: SEQ ID NO: 9, HCDR2: SEQ ID NO: 14 and HCDR3: SEQ ID NO: 17;~~

- ~~(4) HCDR1: SEQ ID NO: 9, HCDR2: SEQ ID NO: 13 and HCDR3: SEQ ID NO: 18;
——and~~
- ~~(5) HCDR1: SEQ ID NO: 9, HCDR2: SEQ ID NO: 13 and HCDR3: SEQ ID NO: 19.~~
- ~~22. The isolated antigen-binding protein according to any one of claims 1-21, which comprises a heavy chain variable region VH, wherein the VH comprises a framework region H FR1, the C terminus of the H FR1 is directly or indirectly linked to the N-terminus of the HCDR1, and the H FR1 comprises an amino acid sequence set forth in SEQ ID NO: 25.~~
- ~~23. The isolated antigen-binding protein according to claim 22, wherein the H FR1 comprises an amino acid sequence set forth in any one of SEQ ID NOs: 7-8.~~
- ~~24. The isolated antigen-binding protein according to any one of claims 22-23, wherein the VH comprises a framework region H FR2, the H FR2 is located between the HCDR1 and the HCDR2, and the H FR2 comprises an amino acid sequence set forth in SEQ ID NO: 26.~~
- ~~25. The isolated antigen-binding protein according to claim 24, wherein the H FR2 comprises an amino acid sequence set forth in any one of SEQ ID NOs: 10-11.~~
- ~~26. The isolated antigen-binding protein according to any one of claims 22-25, wherein the VH comprises a framework region H FR3, the H FR3 is located between the HCDR2 and the HCDR3, and the H FR3 comprises an amino acid sequence set forth in SEQ ID NO: 27.~~
- ~~27. The isolated antigen-binding protein according to claim 26, wherein the H FR3 comprises an amino acid sequence set forth in any one of SEQ ID NO: 15-16.~~
- ~~28. The isolated antigen-binding protein according to any one of claims 22-27, wherein the N-terminus of the H FR4 is linked to the C terminus of the HCDR3, and the H FR4 comprises an amino acid sequence set forth in SEQ ID NO: 28.~~
- ~~29. The isolated antigen-binding protein according to claim 28, wherein the H FR4 comprises an amino acid sequence set forth in any one of SEQ ID NOs: 20-22.~~
- ~~30. The isolated antigen-binding protein according to any one of claims 1-29, which comprises H FR1, H FR2, H FR3 and H FR4, wherein the H FR1 comprises an amino~~

~~acid sequence set forth in SEQ ID NO: 25, the H-FR2 comprises an amino acid sequence set forth in SEQ ID NO: 26, the H-FR3 comprises an amino acid sequence set forth in SEQ ID NO: 27 and the H-FR4 comprises an amino acid sequence set forth in SEQ ID NO: 28.~~

~~31. The isolated antigen-binding protein according to any one of claims 1-30, which comprises H-FR1, H-FR2, H-FR3 and H-FR4, wherein the H-FR1 comprises an amino acid sequence set forth in SEQ ID NO: 7 or SEQ ID NO: 8, the H-FR2 comprises an amino acid sequence set forth in SEQ ID NO: 10 or SEQ ID NO: 11, the H-FR3 comprises an amino acid sequence set forth in SEQ ID NO: 15 or SEQ ID NO: 16, and the H-FR4 comprises an amino acid sequence set forth in any one of SEQ ID NOs: 20-22.~~

326. ~~The antibody or antigen binding fragment according to any one of claims 1 to 5, The isolated antigen-binding protein according to any one of claims 1-31,~~ which comprises H-FR1, H-FR2, H-FR3 and H-FR4, and the H-FR1, H-FR2, H-FR3 and H-FR4 are selected from any one of the following groups of amino acid sequences:

- (1) F-FR1: SEQ ID NO: 7, H-FR2: SEQ ID NO: 10, H-FR3: SEQ ID NO: 15 and H-FR4: SEQ ID NO: 20;
- (2) H-FR1: SEQ ID NO: 8, H-FR2: SEQ ID NO: 11, H-FR3: SEQ ID NO: 16 and H-FR4: SEQ ID NO: 21; and
- (3) H-FR1: SEQ ID NO: 8, H-FR2: SEQ ID NO: 11, H-FR3: SEQ ID NO: 16 and H-FR4: SEQ ID NO: 22.

~~33. The isolated antigen-binding protein according to any one of claims 1-32, which comprises a heavy chain variable region VH, and the VH comprises an amino acid sequence set forth in SEQ ID NO: 29.~~

347. ~~The antibody or antigen binding fragment~~ isolated antigen-binding protein according to claim ~~336~~, wherein the VH comprises an amino acid sequence set forth in any one of SEQ ID NOs: 1-6.

358. One or more isolated nucleic acid molecules encoding the antibody or antigen binding fragment according to any one of claims 1 to 7 ~~the isolated antigen-binding~~

- ~~protein of any one of claims 1-34.~~
369. A vector comprising the nucleic acid molecule of claim ~~35~~8.
- ~~37~~10. A cell comprising the nucleic acid molecule of claim ~~35~~8 or the vector of claim ~~36~~9.
- ~~38~~11. A method for preparing ~~the isolated antigen-binding protein of any one of claims 1-34~~the antibody or antigen binding fragment according to any one of claims 1 to 7, the method comprising culturing the cell of claim ~~37~~10 under such a condition that ~~the isolated antigen-binding protein of any one of claims 1-34~~the antibody or antigen binding fragment according to any one of claims 1 to 7 is expressed.
- ~~39~~12. A pharmaceutical composition comprising ~~the isolated antigen-binding protein of any one of claims 1-34~~the antibody or antigen binding fragment according to any one of claims 1 to 7, the nucleic acid molecule of claim ~~35~~8, the vector of claim ~~36~~9 and/or the cell of claim ~~37~~10, and optionally a pharmaceutically acceptable carrier.
- ~~40~~13. A polypeptide comprising ~~the isolated antigen-binding protein of any one of claims 1-34~~the antibody or antigen binding fragment according to any one of claims 1 to 7.
- ~~41~~14. An immunoconjugate comprising ~~the isolated antigen-binding protein of any one of claims 1-34~~the antibody or antigen binding fragment according to any one of claims 1 to 7 or the polypeptide of claim ~~40~~13.
- ~~42~~15. Use of ~~the isolated antigen-binding protein of any one of claims 1-34~~the antibody or antigen binding fragment according to any one of claims 1 to 7, the nucleic acid molecule of claim ~~35~~8, the vector of claim ~~36~~9, the cell of claim ~~37~~10, the pharmaceutical composition of claim ~~39~~12, the polypeptide of claim ~~40~~13 and/or the immunoconjugate of claim ~~41~~14 in the preparation of a medicament for preventing, alleviating and/or treating tumors, preferably, -
- ~~43. The use according to claim 42, wherein the tumor includes a VEGF-overexpressing tumor, optionally,~~
- ~~44. The use according to claim 42 or 43, wherein the tumor includes a solid tumor and/or a non-solid tumor, optionally,-~~
- ~~45. The use according to any one of claims 42-44, wherein the tumor includes lung cancer, colorectal cancer, breast cancer, kidney cancer, gastric cancer, liver cancer, blastoma,~~

cervical cancer and/or ovarian cancer.

46. ~~A method for preventing, alleviating or treating tumors, the method comprising administering the isolated antigen binding protein of any one of claims 1-34, the nucleic acid molecule of claim 35, the vector of claim 36, the cell of claim 37, the pharmaceutical composition of claim 39, the polypeptide of claim 40 and/or the immunoconjugate of claim 41 to a subject in need thereof.~~
47. ~~The method according to claim 46, wherein the tumor includes a VEGF-overexpressing tumor.~~
48. ~~The method of claim 46 or 47, wherein the tumor includes a solid tumor and/or a non-solid tumor.~~
49. ~~The method according to any one of claims 46-48, wherein the tumor includes lung cancer, colorectal cancer, breast cancer, kidney cancer, gastric cancer, liver cancer, blastoma, cervical cancer and/or ovarian cancer.~~
5016. The isolated antigen binding protein of any one of claims 1-34~~The antibody or antigen binding fragment according to any one of claims 1 to 7,~~ the nucleic acid molecule of claim 35~~8,~~ the vector of claim 36~~9,~~ the cell of claim 37~~10,~~ the pharmaceutical composition of claim 39~~12,~~ the polypeptide of claim 40-~~13~~13 and/or the immunoconjugate of claim 41-~~14~~14 for use in preventing, alleviating or treating tumors, preferably -
51. ~~The isolated antigen binding protein, nucleic acid molecule, vector, cell, pharmaceutical composition, polypeptide and/or immunoconjugate according to claim 50, wherein the tumor includes a VEGF-overexpressing tumor, optionally -~~
52. ~~The isolated antigen binding protein, nucleic acid molecule, vector, cell, pharmaceutical composition, polypeptide and/or immunoconjugate according to claim 50 or 51, wherein the tumor includes a solid tumor and/or a non-solid tumor, optionally -~~
53. ~~The isolated antigen binding protein, nucleic acid molecule, vector, cell, pharmaceutical composition, polypeptide and/or immunoconjugate according to any one of claims 50-52, wherein the tumor includes lung cancer, colorectal cancer, breast cancer, kidney cancer, gastric cancer, liver cancer, blastoma, cervical cancer and/or ovarian cancer.~~

5417. ~~A method for inhibiting the binding of VEGF protein to VEGFR protein, comprising administering~~ Use of the isolated antigen-binding protein of any one of claims 1-34 ~~the antibody or antigen binding fragment according to any one of claims 1 to 7, the nucleic acid molecule of claim 35~~ 8, the vector of claim 36 9, the cell of claim 37 10, the pharmaceutical composition of claim 39 12, the polypeptide of claim 40-13 and/or the immunoconjugate of claim 41 14 in the manufacture of a medicament for inhibiting the binding of VEGF protein to VEGFR protein.