Claims

1. A bispecific binding molecule comprising at least one VEGF-binding component, at least one Ang2-binding component and at least one serum albumin binding component, wherein said Ang2-binding component binds to Ang2 with a potency at least 5,000 times higher than to Ang1 or to Ang4.

2. A bispecific binding molecule of claim 1, wherein said VEGF-binding component comprises at least a variable domain with four framework regions and three complementarity determining regions CDR1, CDR2 and CDR3, respectively, wherein said CDR3 has the amino acid sequence Ser Arg Ala Tyr Xaa Ser Xaa Arg Leu Arg Leu Xaa Xaa Thr Tyr Xaa Tyr as shown in SEQ ID NO: 1, wherein
Xaa at position 5 is Gly or Ala;
Xaa at position 7 is Ser or Gly;
Xaa at position 12 is Gly, Ala or Pro;
Xaa at position 13 is Asp or Gly;
Xaa at position 16 is Asp or Glu; and
wherein said VEGF-binding component is capable of blocking the interaction of human recombinant VEGF165 with the human recombinant VEGFR-2 with an inhibition rate of ≥60%.

3. A bispecific binding molecule of claim 2, wherein said CDR3 has a sequence selected from
SEQ ID NO: 2 SRAYGSSRLRLGDTYDY,
SEQ ID NO: 3 SRAYGSSRLRLADTYDY;
SEQ ID NO: 4 SRAYGSSRLRLADTYEY;
SEQ ID NO: 5 SRAYGSGRLRLADTYDY;
SEQ ID NO: 6 SRAYASSRLRLADTYDY;

SEQ ID NO: 7 SRAYGSSRLRLPDTYDY;
SEQ ID NO: 8 SRAYGSSRLRLPGTYDY.

4. A bispecific binding molecule of claim 3, wherein said VEGF-binding component comprises one or more immunoglobulin single variable domains each containing

   a. a CDR3 with an amino acid sequence selected from a first group of sequences shown in SEQ ID NO: 2 to 8;

   b. a CDR1 and a CDR2 with an amino acid sequences that is contained, as indicated in Table 3, in a sequence selected from a second group of sequences shown in SEQ ID NOs: 9 to 46, wherein said second sequence contains the respective CDR3 in said selected sequence according to a).

5. A bispecific binding molecule of claim 4, wherein said one or more immunoglobulin single variable domains are VHHs.

6. A bispecific binding molecule of claim 5, wherein said one or more VHHs have amino acid sequences selected from the amino acid sequences shown in SEQ ID NOs: 9 - 46.

7. A bispecific binding molecule of claim 6, which comprises one or more VHHs having amino acid sequences selected from SEQ ID NO: 15, SEQ ID NO: 18 and SEQ ID NO: 25.

8. A bispecific binding molecule, the VEGF-binding component of which has been obtained by affinity maturation and/or sequence optimization of a VHH defined in claim 7.

9. A bispecific binding molecule according to claim 8, the VEGF-binding component of which has been obtained by sequence optimization of a VHH having an amino acid sequence shown in SEQ ID NO: 18.

10. A bispecific binding molecule according to claim 9, the VEGF-binding component of which having an amino acid sequence selected from sequences shown in SEQ ID NOs: 47 – 57.

11. A bispecific binding molecule according to claim 5, the VEGF-binding component of which comprising two or more VHHs, which are

a. identical VHHs that are capable of blocking the interaction between recombinant human VEGF and the recombinant human VEGFR-2 with an inhibition rate of ≥ 60% or

b. different VHHs that bind to non-overlapping epitopes of VEGF, wherein at least one VHH is capable of blocking the interaction between recombinant human VEGF and the recombinant human VEGFR-2 with an inhibition rate of ≥ 60% and wherein at least one VHH is capable of blocking said interaction with an inhibition rate of ≤ 60 %.

12. A bispecific binding molecule according to claim 11, wherein said identical VHHs a) are selected from VHHs having amino acid sequences shown in
SEQ ID NOs: 9 – 46 or VHHs that have been obtained by affinity maturation and/or sequence optimization of such VHH.

13. A bispecific binding molecule according to claim 12, wherein said VHH is selected from VHHs having the amino acid shown in SEQ ID NO: 18 or SEQ ID NO: 47 – 57.

14. A bispecific binding molecule according to claim 13 comprising two VHHs each having the amino acid sequence shown in SEQ ID NO: 57.

15. A bispecific binding molecule according to claim 14, wherein

a. said one or more VHHs with an inhibition rate of ≥ 60% are selected from

i. VHHs having an amino acid sequence selected from amino acid sequences shown in SEQ ID NOs: 9 – 46 or

ii. VHHs that have been obtained by affinity maturation and/or sequence optimization of such VHHs, and wherein

b. said one or more VHHs with an inhibition rate of ≤ 60 % are selected from

i. SEQ ID NOs: 58 – 124 or

ii. VHHs that have been obtained by affinity maturation and/or sequence optimization of such VHH.

16. A bispecific binding molecule according to claim 15, wherein two VHHs are contained in polypeptides with amino acid sequences shown in SEQ ID NOs: 128 – 168, separated by linker sequences as indicated in Table 13.

17. A bispecific binding molecule according to claim 16, wherein said VHH a) i. has an amino acid sequence shown in SEQ ID NO: 18 and said VHH b) i. has an amino acid sequence shown in SEQ ID NO: 64.

18. A bispecific binding molecule according to claim 17, wherein said VHHs according to a) ii) are selected from VHHs having an amino acid sequence shown in SEQ ID NOs: 47 – 57 and wherein said VHHs

according to b) ii) are selected from VHHs having an amino acid sequence shown in SEQ ID NOs: 125 – 127.

19. A bispecific binding molecule according to claim 18, comprising two VHHs, one of them having the amino acid shown in SEQ ID NO: 57 and one of them having the amino acid shown in SEQ ID NO: 127.

20. The bispecific binding molecule of any one of claims 1 to 19, comprising an Ang2-binding component comprising at least a variable domain with four framework regions and three complementarity determining regions CDR1, CDR2 and CDR3, respectively, wherein said CDR3 has an amino acid sequence selected from amino acid sequences shown in SEQ IDs NOs: 226, 229, 232, 235, 238, 241, 244, 247, 250, or 253.

21. The bispecific binding molecule of claim 20, the Ang2-binding component of which is an isolated immunoglobulin single variable domain or a polypeptide containing one or more of said immunoglobulin single variable domains, wherein said immunoglobulin single variable domain consists of four framework regions and three complementarity determining regions CDR1, CDR2 and CDR3, respectively, and wherein said CDR3 has an amino acid sequence selected from amino acid sequences shown in SEQ IDs NOs: 226, 229, 232, 235, 238, 241, 244, 247, 250, or 253.

22. The bispecific binding molecule of claim 21, wherein said one or more immunoglobulin single variable domain contain

a. a CDR3 with an amino acid sequence selected from a first group of amino acid sequences shown in SEQ ID NOs: SEQ IDs NOs: 226, 229, 232, 235, 238, 241, 244, 247, 250, or 253 (Table 49);

b. a CDR1 with an amino acid sequences that is contained, as indicated in Table 36-A, 38-A, 41-A, or 45-A, as partial sequence in a sequence selected from a second group of amino acid sequences shown SEQ ID NOs: 224, 227, 230, 233, 236, 239, 242, 245, 248, or 251 (Table 49);

c. a CDR2 with an amino acid sequences that is contained, as indicated in Table 36-A, 38-A, 41-A, or 45-A, as partial sequence in a sequence selected from a second group of amino acid sequences shown SEQ ID NOs:225, 228, 231, 234, 237, 240, 243, 246, 249, or 252 (Table 49).

23. The bispecific binding molecule of any one of claims 20 to 22, wherein said one or more immunoglobulin single variable domains are VHHs.

24. The bispecific binding molecule of claim 23, wherein said one or more VHHs have an amino acid sequence selected from amino acid sequences shown in SEQ ID NOs: 214, 215, 216, 217, 218, 219, 220, 221, 222, or 223.

25. An immunoglobulin single variable domain which has been obtained by affinity maturation of an immunoglobulin single variable domain as defined in claim 22.

26. A VHH which has been obtained by affinity maturation of a VHH as defined in claim 24.

27. An Ang2-binding VHH with an amino acid sequence selected from acid sequences shown in SEQ ID NOs: 214, 215, 216, 217, 218, 219, 220, 221, 222, or 223.

28. An immunoglobulin single variable domain which has been obtained by humanization of a VHH defined in claim 27.

29. An immunoglobulin single variable domain which has been obtained by humanization of an immunoglobulin single variable domain as defined in claim 22.

30. The binding molecule of any one of claim 1 to 29, the serum albumin binding component of which is an isolated immunoglobulin single variable domain or a polypeptide containing one or more of said immunoglobulin single variable domains, wherein said immunoglobulin single variable domain consists of four framework regions and three complementarity determining regions CDR1, CDR2 and CDR3, respectively, and wherein said CDR3 has an amino acid sequence selected from amino acid sequences shown in SEQ ID NOs: 257, 260, 263, 266, 269, 272, or 275.

31. The binding molecule of claim 30, wherein said one or more immunoglobulin single variable domain contain

a. a CDR3 with an amino acid sequence selected from a first group of amino acid sequences shown in SEQ ID NOs: SEQ IDs NOs: 257, 260, 263, 266, 269, 272, or 275;

b. a CDR1 with an amino acid sequences selected from a second group of amino acid sequences shown SEQ ID NOs: 255, 258, 261, 264, 267, 270, or 273;

c. a CDR2 with an amino acid sequences selected from a second group of amino acid sequences shown SEQ ID NOs: 256, 259, 262, 265, 268, 271, or 274.

32. The bispecific binding molecule of claim 30 or 31, wherein said one or more immunoglobulin single variable domains are VHHs.

33. The bispecific binding molecule of claim 32, wherein said one or more VHHs have an amino acid sequence shown in SEQ ID NOs: 98 or 254.

34. The bispecific binding molecule of any one of claims 1 to 33 having the amino acid sequence selected from amino acid sequences shown in SEQ ID NOs: 180-213.

35. A nucleic acid molecule encoding a bispecific binding molecule of any one of claims 1 to 34 or a vector containing same.

36. A host cell containing a nucleic acid molecule of claim 35.

37. A pharmaceutical composition containing at least one bispecific binding molecule of any one of claims 1 to 34 as the active ingredient.

38. The pharmaceutical composition of claim 37 for the treatment of a disease that is associated with VEGF- and/or Ang2-mediated effects on angiogenesis.

39. The pharmaceutical composition of claim 38 for the treatment of cancer and cancerous diseases.

40. The pharmaceutical composition of claim 38 for the treatment of eye diseases.

Claims

1. A bispecific binding molecule comprising
- at least one VEGF-binding component,
- at least one serum albumin binding component, and
- at least one Ang2-binding component, wherein said Ang2-binding component binds to Ang2 with a potency at least 5,000 times higher than to Ang1 or to Ang4, and

wherein said VEGF-, serum albumin and Ang2-binding components are immunoglobulin single variable domains, each immunoglobulin single variable domain consisting of four framework regions and three complementarity determining regions (CDRs), and wherein said bispecific binding molecule is selected from the group consisting of bispecific binding molecules having
(i) the CDR sequences as present in VEGFANGBII00022 (SEQ ID NO: 207),
(ii) the CDR sequences as present in VEGFANGBII00025 (SEQ ID NO: 210), and
(iii) the CDR sequences as present in VEGFANGBII00028 (SEQ ID NO: 213).

2. The bispecific binding molecule of claim 1, wherein said immunoglobulin single variable domains are VHHs.

3. The bispecific binding molecule of claim 2, selected from the group consisting of bispecific binding molecules (i) to (iii),
the molecules (i) comprising
- a VEGF binding domain comprising the CDRs 1 to 3 of the VEGFBII00038 VHH domain (SEQ ID NO:57),
- the Alb11 VHH domain (SEQ ID NO: 254) as the serum albumin binding component, and
- the ANGBII00938 VHH domain (SEQ ID NO: 222) as the Ang2-binding domain, in this order;
the molecules (ii) comprising
- a VEGF binding domain comprising the CDRs 1 to 3 of the VEGFBII00038 VHH domain (SEQ ID NO:57),

- the Alb11 VHH domain (SEQ ID NO: 254) as the serum albumin binding component, and
- two ANGBII00921 VHH domains (SEQ ID NO: 220) as the Ang2-binding domains, in this order;
and
the molecules (iii) comprising
- a VEGF binding domain comprising the CDRs 1 to 3 of the VEGFBII00038 VHH domain (SEQ ID NO:57),
- the Alb11 VHH domain (SEQ ID NO: 254) as the serum albumin binding component, and
- two ANGBII00956 VHH domains (SEQ ID NO: 223) as the Ang2-binding domains,
in this order.

4. The bispecific binding molecule of claim 3, selected from the group consisting of
(i) VEGFANGBII00022 (SEQ ID NO: 207),
(ii) VEGFANGBII00025 (SEQ ID NO: 210), and
(iii) VEGFANGBII00028 (SEQ ID NO: 213).

5. A nucleic acid molecule encoding a bispecific binding molecule of any one of claims 1 to 4.

6. A vector containing a nucleic acid molecule of claim 5.

7. A host cell containing a nucleic acid molecule of claim 5 or a vector of claim 6.

8. A pharmaceutical composition containing at least one bispecific binding molecule of any one of claims 1 to 4 as the active ingredient.

9. The pharmaceutical composition of claim 8 for the treatment of a disease that is associated with VEGF- and/or Ang2-mediated effects on angiogenesis.

10. The pharmaceutical composition of claim 8 or 9 for the treatment of cancer and cancerous diseases.

11. The pharmaceutical composition of claim 8 or 9 for the treatment of eye diseases.