

collaterals. There is also the serious concern of systemic exposure of these drugs in elderly and/or diabetic patients where new vessel growth may be required in ischemic brains, hearts or limbs.

5 Typically for ocular diseases via intravitreal application smaller antibody fragments like Fab or Fab₂ are often used as they have a low serum half-life and the risk of systemic toxicities is lower. However this smaller fragments typically have also lower intravitreal half-lives (e.g. due to the faster diffusion into serum) and have to be dosed typically more often.

10 Multispecific antibodies with a domain replacement/exchange in one binding arm (CrossMabVH-VL) are described in detail in WO 2009/080252 and Schaefer, W. et al, Proc. Natl. Acad. Sci. USA, 108 (2011) 11187-11191 (which are incorporated as reference herein). They clearly reduce the byproducts caused by the mismatch of a light chain against a first antigen with the wrong heavy chain against the second antigen (compared to approaches without such domain exchange). However their
15 preparation is not completely free of side products. The main side product is based on a Bence-Jones-type interaction. See also Schaefer, W. et al, Proc. Natl. Acad. Sci. USA, 108 (2011) 11187-11191; in Figure SII of the Supplement).

In WO 2011/117329 bispecific, bivalent anti-VEGF/anti-ANG2 antibodies are reported. Human FcRn-binding modified antibodies and methods of use are
20 reported in WO 2014/177460. Kienast, Y., et al. (Clin. Canc. Res. 19 (2013) 6730-6740) reported Ang-2-VEGF-A CrossMab as a novel bispecific human IgG1 antibody blocking VEGF-A and Ang-2 functions simultaneously, mediates potent antitumor, antiangiogenic, and anti-metastatic efficacy.

SUMMARY

25 The disclosure provides novel bispecific antibodies and methods of using the same:

Herein is reported a bispecific antibody that specifically binds to two different antigens selected from the group consisting of human ANG2, human VEGF, human IL-1beta and human PDGF-B.

In one embodiment the antibody is not an anti-ANG2/VEGF antibody.

30 In the disclosure the antibody specifically binds to human IL-1beta and comprises

a) a heavy chain variable domain comprising

(a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 02,
(b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO:
04, and (c) a HVR-H3 comprising the amino acid sequence of SEQ
ID NO: 05, or

(a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 07,
(b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO:
08, and (c) a HVR-H3 comprising the amino acid sequence of SEQ
ID NO: 10, or

(a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12,
(b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO:
13, and (c) a HVR-H3 comprising the amino acid sequence of SEQ
ID NO: 15,

and

a light chain variable domain comprising (a) a HVR-L1 comprising the
amino acid sequence of SEQ ID NO: 17; (b) a HVR-L2 comprising the
amino acid sequence of SEQ ID NO: 18; and (c) HVR-L3 comprising
the amino acid sequence of SEQ ID NO: 19,

or

a) a heavy chain variable domain comprising a HVR-H1 comprising the
amino acid sequence of SEQ ID NO: 21, (b) a HVR-H2 comprising the
amino acid sequence of SEQ ID NO: 22, and (c) a HVR-H3 comprising
the amino acid sequence of SEQ ID NO: 24, and a light chain variable
domain comprising (a) a HVR-L1 comprising the amino acid sequence
of SEQ ID NO: 26; (b) a HVR-L2 comprising the amino acid sequence
of SEQ ID NO: 27; and (c) HVR-L3 comprising the amino acid
sequence of SEQ ID NO: 28.

One aspect as reported herein is a bispecific antibody that specifically binds to human IL-1beta, comprising

a) a heavy chain variable domain comprising hypervariable regions selected from the group of

5 • a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 02, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 03, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 05,

10 • a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 07, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 08, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 10,

15 • a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15;

and

a light chain variable domain comprising

20 • a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 17; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 18; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 19;

or

b) a heavy chain variable domain comprising

25 • a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 24,

and a light chain variable domain comprising

- a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 26; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 27; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 28,

5 wherein the bispecific antibody specifically binds to a second antigen selected from the group consisting of human ANG2, human VEGF and human PDGF-B; and

wherein the antibody is of human subclass IgG1 or of human subclass IgG4; and

wherein the antibody comprises the mutations I253A, H310A, and H435A, wherein the numbering is according to the EU index of Kabat.

10 In the disclosure the antibody specifically binds to human PDGF-B and comprises

- a) a heavy chain variable domain comprising (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 30, (b) a HVR-H2 comprising

the amino acid sequence of SEQ ID NO: 31, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 33, and a light chain variable domain comprising (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 35; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 36; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 37,

or

b) a heavy chain variable domain comprising (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 39, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 40, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 42, and a light chain variable domain comprising (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 44; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 45; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 46,

or

c) a heavy chain variable domain comprising (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 48, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 49, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 51, and a light chain variable domain comprising (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

One aspect as reported herein is a bispecific antibody that specifically binds to human PDGF-B, comprising

a) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 30, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 33, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 35; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 36; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 37;

or

- 5 b) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 39, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 40, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 42, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 44; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 45; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 46;

or

- 10 c) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 48, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 49, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 51, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55;

wherein the bispecific antibody specifically binds to a second antigen selected from the group consisting of human ANG2, human VEGF and human IL-1beta;

wherein the antibody is of human subclass IgG1 or of human subclass IgG4; and

- 20 wherein the antibody comprises the mutations I253A, H310A, and H435A, wherein the numbering is according to the EU index of Kabat.

In one embodiment the antibody specifically binds to human ANG2 and comprises

- 25 a) a heavy chain variable domain comprising (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 57, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 60, and a light chain variable domain comprising (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 62; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 63; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 64,
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- ii) H310A, H433A, and Y436A, or
- iii) L251D, L314D, and L432D, or
- iv) combinations of i) to iii)

in the first Fc-region polypeptide and in the second Fc-region polypeptide.

- 5 One aspect as reported herein is a pharmaceutical formulation comprising an antibody as reported herein and optionally a pharmaceutically acceptable carrier.

One aspect as reported herein is the antibody as reported herein for use as a medicament.

- 10 One aspect as reported herein is the antibody as reported herein for use in the treatment of an ocular vascular disease.

One aspect as reported herein is the antibody as reported herein for use in the treatment of an eye disease.

One aspect as reported herein is the use of the antibody as reported herein in the manufacture of a medicament.

- 15 In one embodiment the medicament is for the treatment of an ocular vascular disease, preferably for the treatment of macular degeneration.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

I. DEFINITIONS

- 20 An “acceptor human framework” for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid
- 25 sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

5 “Affinity” refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (k_d).

Patent Claims

1. A bispecific antibody that specifically binds to human IL-1beta, comprising

a) a heavy chain variable domain comprising hypervariable regions selected from the group of

- 5 • a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 02, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 03, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 05,
- 10 • a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 07, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 08, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 10,
- 15 • a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15;

and

a light chain variable domain comprising

- 20 • a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 17; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 18; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 19;

or

b) a heavy chain variable domain comprising

- 25 • a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 24,

and a light chain variable domain comprising

- a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 26; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 27; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 28,

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wherein the bispecific antibody specifically binds to a second antigen selected from the group consisting of human ANG2, human VEGF and human PDGF-B; and

wherein the antibody is of human subclass IgG1 or of human subclass IgG4; and

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wherein the antibody comprises the mutations I253A, H310A, and H435A, wherein the numbering is according to the EU index of Kabat.

2. A bispecific antibody that specifically binds to human PDGF-B, comprising

5 a) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 30, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 33, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 35; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 36; and HVR-L3 comprising the amino acid sequence of
10 SEQ ID NO: 37;

or

15 b) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 39, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 40, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 42, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 44; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 45; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 46;

20 or

25 c) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 48, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 49, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 51, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55;

30 wherein the bispecific antibody specifically binds to a second antigen selected from the group consisting of human ANG2, human VEGF and human IL-1beta;

wherein the antibody is of human subclass IgG1 or of human subclass IgG4; and

wherein the antibody comprises the mutations I253A, H310A, and H435A, wherein the numbering is according to the EU index of Kabat.

- 5 3. The bispecific antibody according to any one of claims 1 or 2, wherein the antibody specifically binds to human ANG-2, wherein the antibody comprises

5 a) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 57, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 60, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 62; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 63; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 64,

or

10 b) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 66, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 67, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 69, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of
15 SEQ ID NO: 71; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 72; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 73,

or

20 c) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 75, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 76, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 78, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 80; a HVR-L2 comprising the amino acid sequence of
25 SEQ ID NO: 81; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 82,

or

30 d) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 84, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 85, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 87, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 89; a HVR-L2 comprising the amino

acid sequence of SEQ ID NO: 90; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 91.

4. The bispecific antibody according to any one of claims 1 or 2 that further specifically binds to human VEGF and further comprises
 - 5 a) the HVR-H1, HVR-H2 and HVR-H3 as contained in the heavy chain variable domain of SEQ ID NO: 107 and the HVR-L1, HVR-L2 and HVR-L3 as contained in the heavy chain variable domain of SEQ ID NO: 108,

or
 - 10 b) the HVR-H1, HVR-H2 and HVR-H3 as contained in the heavy chain variable domain of SEQ ID NO: 109 and the HVR-L1, HVR-L2 and HVR-L3 as contained in the heavy chain variable domain of SEQ ID NO: 110.
5. The bispecific antibody according to claim 1 that further specifically binds to human PDGF-B, comprising
- 15

a) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 30, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 31, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 33, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 35; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 36; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 37,

or

b) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 39, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 40, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 42, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 44; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 45; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 46,

or

- 5 c) a heavy chain variable domain comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 48, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 49, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 51, and a light chain variable domain comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

- 10 6. The antibody according to any one of claims 1 to 5, wherein the antibody is a bivalent, bispecific antibody comprising

- a) a first light chain and a first heavy chain of an antibody specifically binding to a first antigen, and
- 15 b) a second light chain and a second heavy chain of an antibody specifically binding to a second antigen, wherein the variable domains VL and VH of the second light chain and the second heavy chain are replaced by each other.

7. The antibody according to claim 6, wherein the antibody comprises
- 20 i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted
- 25 independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index),

or

- 30 ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein

- 5 v) the first Fc-region polypeptide is a human IgG1 Fc-region polypeptide with the mutations L234A, L235A, P329G, S354C, T366W and the second Fc-region polypeptide is a human IgG1 Fc-region polypeptide with the mutations L234A, L235A, P329G, Y349C, T366S, L368A, Y407V, or
- 10 vi) the first Fc-region polypeptide is a human IgG4 Fc-region polypeptide and the second Fc-region polypeptide is a human IgG4 Fc-region polypeptide, or
- 10 vii) the first Fc-region polypeptide is a human IgG4 Fc-region polypeptide with the mutations S228P, L235E and the second Fc-region polypeptide is a human IgG4 Fc-region polypeptide with the mutations S228P, L235E, or
- 15 viii) the first Fc-region polypeptide is a human IgG4 Fc-region polypeptide with the mutations S228P, L235E, P329G and the second Fc-region polypeptide is a human IgG4 Fc-region polypeptide with the mutations S228P, L235E, P329G, or
- 20 ix) the first Fc-region polypeptide is a human IgG4 Fc-region polypeptide with the mutations S228P, L235E, S354C, T366W and the second Fc-region polypeptide is a human IgG4 Fc-region polypeptide with the mutations S228P, L235E, Y349C, T366S, L368A, Y407V, or
- 25 x) the first Fc-region polypeptide is a human IgG4 Fc-region polypeptide with the mutations S228P, L235E, P329G, S354C, T366W and the second Fc-region polypeptide is a human IgG4 Fc-region polypeptide with the mutations S228P, L235E, P329G, Y349C, T366S, L368A, Y407V.

10. A pharmaceutical formulation comprising an antibody according to any one of claims 1 to 9 and optionally a pharmaceutically acceptable carrier.
11. The antibody according to any one of claims 1 to 9 for use as a medicament.
12. The antibody according to any one of claims 1 to 9 for use in the treatment of an ocular vascular disease.
13. The antibody according to any one of claims 1 to 9 for use in the treatment of an eye disease.
14. Use of the antibody according to any one of claims 1 to 9 in the manufacture of a medicament.
15. The use according to claim 14, wherein the medicament is for the treatment of an ocular vascular disease.
16. The use according to claim 14, wherein the medicament is for the treatment of an eye disease.