

elderly and/or diabetic patients where new vessel growth may be required in ischemic brains, hearts or limbs.

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.

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 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 S11 of the Supplement).

In WO 2014/072876 platelet-derived growth factor B specific antibodies and compositions and uses thereof are reported. Multivalent antibody materials and methods for VEGF/PDGF family of growth factors are reported in WO 2005/087812. Vassbotn, F.S., et al. (Biochim. Biophys. Acta. Mol. Cell Res. 1054 (1990) 246-249) report a monoclonal antibody against PDGF B-chain inhibits PDGF-induced DNA synthesis in C3H fibroblasts and prevents binding of PDGF to its receptor.

SUMMARY

The disclosure provides anti-PDGF-B antibodies and methods of using the same. In specific embodiments the antibody is a humanized antibody.

As reported herein is an isolated antibody that binds to PDGF-B, wherein the antibody is a humanized variant of a murine antibody whereby the murine antibody comprises a heavy chain variable domain of SEQ ID NO: 01 and a light chain variable domain of SEQ ID NO: 06.

In one embodiment the humanized antibody further comprises in the heavy chain variable domain at position 27 a tyrosine amino acid

residue, at position 40 an arginine amino acid residue, at position 43 a histidine amino acid residue and at position 73 a tryptophan amino acid residue (numbering according to Kabat).

5 In one embodiment the humanized antibody further comprises in the heavy chain variable domain at position 78 an alanine amino acid residue (numbering according to Kabat).

10 In one embodiment the humanized antibody further comprises in the heavy chain variable domain at position 43 a histidine amino acid residue, at position 71 an alanine amino acid residue, and at position 78 an alanine amino acid residue (numbering according to Kabat).

In one embodiment the humanized antibody further comprises in the light chain variable domain at position 43 the amino acid residue proline, and at position 68 the amino acid residue arginine (numbering according to Kabat).

15 In one embodiment of all aspects as reported herein the humanized antibody further comprises in the light chain variable domain at position 43 the amino acid residue proline (numbering according to Kabat).

20 As reported herein is an antibody that specifically binds to human PDGF-B, wherein the antibody comprises (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: 03, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 05.

25 In one embodiment the antibody further comprises (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 07; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 08; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 09.

30 As reported herein is an antibody that specifically binds the same epitope of human PDGF-B as an antibody 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, (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 05, (d) a HVR-L1 comprising the amino acid sequence of

SEQ ID NO: 07; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 08; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 09.

As reported herein is an antibody that specifically binds to human PDGF-B, wherein the antibody comprises

- 5 a) (i) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
 (ii) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 12,
 and (iii) a HVR-H3 comprising the amino acid sequence of SEQ ID NO:
 14, or
- 10 b) (i) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 16,
 (ii) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 17,
 and (iii) a HVR-H3 comprising the amino acid sequence of SEQ ID NO:
 19, or
- 15 c) (i) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21,
 (ii) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22,
 and (iii) a HVR-H3 comprising the amino acid sequence of SEQ ID NO:
 24, or
- 20 d) (i) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 26,
 (ii) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 27,
 and (iii) a HVR-H3 comprising the amino acid sequence of SEQ ID NO:
 29, or
- e) (i) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 31,
 (ii) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 32,
 and (iii) a HVR-H3 comprising the amino acid sequence of SEQ ID NO:
 34, or
- 25 f) (i) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 36,
 (ii) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 37
 and (iii) a HVR-H3 comprising the amino acid sequence of SEQ ID NO:
 39.

In one embodiment the antibody further comprises

- 30 a) (vi) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 41,
 (v) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 42,
 (vi) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 43,
 or
- 35 b) (vi) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 45,
 (v) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 46,

- (vi) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 47,
or
- c) (vi) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 49,
(v) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 50,
5 (vi) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 51,
or
- d) (vi) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53,
(v) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54,
(vi) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

10 In one embodiment the antibody comprises

- a) a variable heavy chain domain comprising the amino acid sequence of
SEQ ID NO: 10, or
- b) a variable heavy chain domain comprising the amino acid sequence of
SEQ ID NO: 15, or
- 15 c) a variable heavy chain domain comprising the amino acid sequence of
SEQ ID NO: 20, or
- d) a variable heavy chain domain comprising the amino acid sequence of
SEQ ID NO: 25, or
- e) a variable heavy chain domain comprising the amino acid sequence of
20 SEQ ID NO: 30, or
- f) a variable heavy chain domain comprising the amino acid sequence of
SEQ ID NO: 35.

In one embodiment the antibody further comprises

- a) a variable light chain domain comprising the amino acid sequence of
25 SEQ ID NO: 40, or
- b) a variable light chain domain comprising the amino acid sequence of
SEQ ID NO: 44, or
- c) a variable light chain domain comprising the amino acid sequence of
SEQ ID NO: 48, or
- 30 d) a variable light chain domain comprising the amino acid sequence of
SEQ ID NO: 52.

As reported herein is an antibody that specifically binds to human PDGF-B,
wherein the antibody comprises (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.

5 In one embodiment the antibody further comprises (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 62; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 63; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 64.

10 As reported herein is an antibody that specifically binds to human PDGF-B, wherein the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 66, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 67, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 69.

15 In one embodiment the antibody further comprises (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 71; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 72; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 73.

20 As reported herein is an antibody that specifically binds to human PDGF-B, wherein the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 75, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 76, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 78.

In one embodiment the antibody further comprises (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 80; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 81; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 82.

25 As reported herein is an antibody that specifically binds to human PDGF-B, wherein the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 84, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 85, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 87.

30 In one embodiment the antibody further comprises (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 89; (e) a HVR-L2 comprising the amino acid

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

As reported herein is an antibody that specifically binds to human PDGF-B, wherein the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 93, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 94, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 96.

The antibody further comprises (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 98; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 99; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 100.

One aspect as reported herein is an antibody that specifically binds to human PDGF-B, wherein the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 93, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 94, (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 96, (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 98, (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 99, and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 100.

As reported herein is an antibody that specifically binds to human PDGF-B, wherein the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 102, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 103, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 105.

In one embodiment the antibody further comprises (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 107; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 108; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 109.

As reported herein is an antibody comprising a heavy chain variable domain amino acid sequence of sequence of SEQ ID NO: 10 and a light chain variable domain amino acid sequence selected from the group of SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 48 and SEQ ID NO: 52.

As reported herein is an antibody comprising a heavy chain variable domain amino acid sequence of sequence of SEQ ID NO: 15 and a light chain variable domain amino acid sequence selected from the group of SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 48 and SEQ ID NO: 52.

- 5 As reported herein is an antibody comprising a heavy chain variable domain amino acid sequence of sequence of SEQ ID NO: 20 and a light chain variable domain amino acid sequence selected from the group of SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 48 and SEQ ID NO: 52.

As reported herein is an antibody comprising a heavy chain variable domain amino acid sequence of sequence of SEQ ID NO: 25 and a light chain variable domain amino acid sequence selected from the group of SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 48 and SEQ ID NO: 52.

5 As reported herein is an antibody comprising a heavy chain variable domain amino acid sequence of sequence of SEQ ID NO: 30 and a light chain variable domain amino acid sequence selected from the group of SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 48 and SEQ ID NO: 52.

10 As reported herein is an antibody comprising a heavy chain variable domain amino acid sequence of sequence of SEQ ID NO: 35 and a light chain variable domain amino acid sequence selected from the group of SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 48 and SEQ ID NO: 52.

As reported herein is an antibody specifically binding to human PDGF-BB and human PDGF-AB but not binding to human PDGF-AA.

15 In one embodiment the antibody comprises (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, (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 60, (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 62; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 63;
20 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 64.

In one embodiment the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 66, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 67, (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 69, (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 71; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 72;
25 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 73.

In one embodiment the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 75, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 76, (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 78, (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 80; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 81;
30 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 82.

In one embodiment the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 84, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 85, (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 87, (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 89; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 90; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 91.

In one embodiment the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 93, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 94, (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 96, (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 98; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 99; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 100.

In one embodiment the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 102, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 103, (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 105, (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 107; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 108; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 109.

As reported herein is an antibody specifically binding to human PDGF-BB and PDGF-AB and PDGF-AA.

In one embodiment the antibody comprises (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: 03, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 05.

In one embodiment the antibody further comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 07; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 08; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 09.

In one embodiment the antibody is of the human subclass IgG1 or the human subclass IgG4.

In one embodiment the antibody is of the human subclass IgG1 with a kappa light chain.

In one embodiment the antibody is a monoclonal antibody.

In one embodiment the antibody is a bispecific antibody.

In one embodiment the antibody specifically binds to human PDGF-B but does not bind to human PDGF-C.

5 In one embodiment the antibody specifically binds to human, rat, mouse and cynomolgus PDGF-B.

In one embodiment the antibody inhibits the biological activity of human PDGF-B by inhibiting the binding of PDGF-B to its receptor.

10 In one embodiment the antibody inhibits the biological activity of human PDGF-B by inhibiting the cell migration.

In one embodiment the antibody inhibits the biological activity of human PDGF-B by inhibiting PDGF-B receptor phosphorylation.

In one embodiment the antibody inhibits the biological activity of human PDGF-B by inhibiting the cell proliferation.

15 As reported herein is an (isolated) nucleic acid encoding the antibody as reported herein.

As reported herein is a host cell comprising the nucleic acid as reported herein.

20 As reported herein is a method of producing an antibody as reported herein comprising culturing the host cell as reported herein to produce the antibody and recovering the antibody from the host cell or the cultivation medium.

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

25 In one embodiment the pharmaceutical formulation further comprises an additional therapeutic agent. In one embodiment the additional therapeutic agent is an anti-ANG2 antibody or an anti-VEGF antibody.

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

As reported herein is the antibody as reported herein for use in treating on ocular vascular disease, preferably for use in treating macular degeneration.

5 The antibody as reported herein for use in inhibiting the interaction between PDGF-B and its receptor.

The antibody as reported herein for use in inhibiting cell migration.

The antibody as reported herein for use in inhibiting proliferation.

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

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

In one embodiment the medicament is for inhibiting the interaction between PDGF-B and its receptor.

15 As reported herein is a method of treating an individual having on ocular vascular disease, preferably macular degeneration, comprising administering to the individual an effective amount of the antibody as reported herein.

20 As reported herein is a method of inhibiting the interaction between PDGF-B and its receptor in an individual comprising administering to the individual an effective amount of the antibody as reported herein to inhibiting the interaction between PDGF-B and its receptor.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

I. DEFINITIONS

25 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

Patent Claims

1. An antibody that specifically binds to human PDGF-B, wherein the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 93, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 94, (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 96, (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 98, (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 99, and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 100.
2. The antibody according to claim 1 wherein the antibody comprises a heavy chain variable domain that has the amino acid sequence of SEQ ID NO: 92 and a light chain variable domain that has the amino acid sequence of SEQ ID NO: 97.
3. The antibody according to any one of claim 1 or 2, wherein the antibody is a bispecific antibody.
4. The antibody according to claim 3, wherein the bispecific antibody comprises
 - a) a first light chain and a first heavy chain of an antibody specifically binding to a first antigen, and
 - b) a second light chain and a second heavy chain of an antibody specifically binding to a second antigen, wherein the constant domains CL and CH1 of the second light chain and the second heavy chain are replaced by each other,

wherein the first antigen or the second antigen is human PDGF-B.

5. The antibody according to claim 4, wherein the antibody is a bivalent antibody.
- 5 6. The antibody according to claim 4 or 5 comprising CH3 domains that are altered to increase the heterodimerization of both heavy chains containing these two CH3 domains, wherein one CH3 domain comprises a T366W mutation and the other CH3 domain comprises T366S, L368A, Y407V mutations, wherein all numberings are according to the Kabat EU index.
- 10 7. The antibody according to claim 4 or 5 comprising CH3 domains that are altered to increase the heterodimerization of both heavy chains containing these two CH3 domains, wherein one CH3 domain comprises Y349C and T366W mutations and the other CH3 domain comprises E356C, T366S, L368A and Y407V mutations, wherein all numberings are according to the Kabat EU index.
- 15 8. The bispecific antibody according to any one of claims 3 to 7, wherein the antibody specifically binds to human PDGF-B and VEGF.
9. The bispecific antibody according to any one of claims 3 to 7, wherein the antibody specifically binds to human PDGF-B and ANG2.
- 20 10. The antibody according to any one of claims 1 to 9, wherein the antibody is of the human subclass IgG1 or the human subclass IgG4.
11. The antibody according to any one of claims 1 to 10, wherein the antibody blocks the biological activity of human PDGF-B by inhibiting the binding of human PDGF-B to human PDGF receptor.
- 25 12. A pharmaceutical formulation comprising an antibody according to any one of claims 1 to 11 and optionally a pharmaceutically acceptable carrier.
13. The pharmaceutical formulation according to claim 12, further comprising an additional therapeutic agent selected from an anti-ANG2 antibody and an anti-VEGF antibody.

14. The antibody according to any one of claims 1 to 11 for use as a medicament.
15. Use of the antibody according to any one of claims 1 to 11 in the manufacture of a medicament.

16. The use according to claim 15, wherein the medicament is for the treatment of an ocular vascular disease, preferably for the treatment of macular degeneration.
 17. The use according to claim 15 or 16, wherein the medicament is for inhibiting the interaction between PDGF-B and the PDGF-B receptor.
- 5