

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 S1I of the Supplement).

In WO 2010/040508 bispecific anti-VEGF/anti-ANG-2 antibodies are reported. Human FcRn-binding modified antibodies and methods of use are reported in WO 2014/177460. Thomas, M., et al. reported a novel angiopoietin-2 selective fully human antibody with potent anti-tumoral and anti-angiogenic efficacy and superior side effect profile compared to pan-angiopoietin-1/-2 inhibitors (PLOS One 8 (2013) E54923). Papadopoulos, K. P., et al. reported a phase I first-in-human study of REGN910 (SAR307746), a fully human and selective angiopoietin-2 (Ang2) monoclonal antibody (MAb), in patients with advanced solid tumor malignancies (abstract 2517, ASCO Annual Meeting 2013).

SUMMARY

The disclosure provides anti-ANG2 antibodies and methods of using the same. In specific embodiments the antibody is an affinity matured antibody.

As reported herein is an antibody that specifically binds to human ANG2, wherein the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23.

One aspect as reported herein is an antibody that specifically binds to human ANG2, wherein the antibody comprises a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; and either a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27, or a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 34; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 35; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 36.

In one embodiment the antibody further comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27.

As reported herein is an antibody that specifically binds to human ANG2, wherein the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 29, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 30, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32.

In one embodiment the antibody further comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 34; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 35; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 36.

As reported herein is an antibody that specifically binds to human ANG2, wherein the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 38, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 41.

In one embodiment the antibody further comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 43; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 44; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 45.

In one embodiment the antibody comprises a heavy chain variable domain that has the amino acid sequence of SEQ ID NO: 19 and a light chain variable domain that has the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 33.

As reported herein is an antibody that specifically binds to human ANG2, wherein the antibody

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- i) comprises a heavy chain variable domain that has a sequence identity to SEQ ID NO: 19 of more than 70 %, or 85 %, or 90 %, or 95 %, and a light chain variable domain that has a sequence identity to SEQ ID NO: 6 of more than 70 %, or 85 %, or 90 %, or 95 %,

- 5 ii) has in the heavy chain variable domain at position 28 the amino acid residue asparagine (N), at position 30 the amino acid residue alanine (A), at position 100b the amino acid residue proline (P) and at position 100j the amino acid residue alanine (A) and in the light chain variable domain at position 51 the amino acid residue threonine (T) (numbering according to Kabat), and
- 10 iii) the antibody has a lower EC₅₀ value for the inhibition of the binding of ANG2 to its receptor Tie2 in a cell based assay using HEK293 cells stably expressing human Tie2 determined using a Tie2 phosphorylation ELISA compared to an antibody comprising a heavy chain variable domain that has the sequence of SEQ ID NO: 19 and a light chain variable domain that has the sequence of SEQ ID NO: 6 or SEQ ID NO: 33.

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

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

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

In one embodiment the antibody is a monoclonal antibody.

- 20 As reported herein is an antibody comprising a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 24.

As reported herein is an antibody comprising a VH sequence of SEQ ID NO: 28 and a VL sequence of SEQ ID NO: 33.

- 25 As reported herein is an antibody comprising a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 42.

In one embodiment the antibody is a bispecific antibody.

In one embodiment the humanized antibody blocks the biological activity of human ANG2 by inhibiting the binding of human ANG2 to the Tie2 receptors.

As reported herein is an antibody Fab fragment that specifically binds to human ANG2, wherein the antibody Fab fragment comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23.

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In one embodiment the antibody Fab fragment further comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27.

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As reported herein is an antibody Fab fragment that specifically binds to human ANG2, wherein the antibody Fab fragment comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 29, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 30, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32.

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In one embodiment the antibody Fab fragment further comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 34; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 35; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 36.

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As reported herein is an antibody Fab fragment that specifically binds to human ANG2, wherein the antibody Fab fragment comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 38, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 41.

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In one embodiment the antibody Fab fragment further comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 43; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 44; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 45.

As reported herein is an antibody Fab fragment comprising a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 24.

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As reported herein is an antibody Fab fragment comprising a VH sequence of SEQ ID NO: 28 and a VL sequence of SEQ ID NO: 33.

As reported herein is an antibody Fab fragment comprising a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 42.

5 As reported herein is a scFv antibody fragment that specifically binds to human ANG2, wherein the scFv antibody fragment comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23.

10 In one embodiment the scFv antibody fragment further comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27.

15 As reported herein is a scFv antibody fragment that specifically binds to human ANG2, wherein the scFv antibody fragment comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 29, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 30, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32.

20 In one embodiment the scFv antibody fragment further comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 34; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 35; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 36.

25 As reported herein is a scFv antibody fragment that specifically binds to human ANG2, wherein the scFv antibody fragment comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 38, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 41.

In one embodiment the scFv antibody fragment further comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 43; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 44; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 45.

30 As reported herein is a scFv antibody fragment comprising a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 24.

As reported herein is a scFv antibody fragment comprising a VH sequence of SEQ ID NO: 28 and a VL sequence of SEQ ID NO: 33.

As reported herein is a scFv antibody fragment comprising a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 42.

5 As reported herein is an anti-ANG2 antibody that interacts with at least 90 % of the residues of ANG2 as an antibody as reported herein when determined by X-ray crystallography.

10 As reported herein is an anti-ANG2 antibody that interacts with at least the same residues of ANG2 as an antibody as reported herein when determined by X-ray crystallography.

As reported herein is an anti-ANG2 antibody that interacts with at least 90 % of the residues of ANG2 as an antibody as reported herein when determined by X-ray crystallography and inhibits the binding of ANG2 to its receptor.

15 As reported herein is an anti-ANG2 antibody that interacts with at least the same residues of ANG2 as an antibody as reported herein when determined by X-ray crystallography and inhibits the binding of ANG2 to its receptor.

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-VEGF antibody, an anti-IL-1beta antibody or an anti-PDGF-B 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 the treatment of vascular diseases.

As reported herein is a pharmaceutical composition for the treatment of vascular diseases.

- 5 One aspect as reported herein is the use of an antibody as reported herein for the manufacture of a medicament. The medicament may be for the treatment of vascular diseases.

- 10 As reported herein is a method of treatment of a patient suffering from a vascular disease by administering an antibody as reported herein to a patient in the need of such treatment.

As reported herein is the antibody as reported herein for use in treating a vascular disease, preferably for use in treating cancer.

One embodiment is the antibody as reported herein for the treatment of cancer.

As reported herein is for use as a medicament for use in the treatment of cancer.

- 15 As reported herein is the use of an antibody as reported herein for the manufacture of a medicament for the treatment of cancer.

As reported herein is a method of treatment of a patient suffering from cancer by administering an antibody as reported herein to a patient in the need of such treatment.

- 20 As reported herein is for use as a medicament for the prevention of metastasis.

As reported herein is the use of an antibody as reported herein for the manufacture of a medicament for the prevention of metastasis.

- 25 As reported herein is a method of prevention of metastasis in a patient suffering from primary cancer by administering an antibody as reported herein to a patient in the need of such preventive treatment.

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

The antibody as reported herein for use in inhibiting the interaction between ANG2 and the Tie2 receptor.

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

5 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 ANG2 and the Tie2 receptor.

10 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.

15 As reported herein is a method of inhibiting the interaction between ANG2 and the Tie2 receptor in an individual comprising administering to the individual an effective amount of the antibody as reported herein to inhibiting the interaction between ANG2 and the Tie2 receptor.

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 sequence thereof, or it may contain amino acid sequence changes. In some
25 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.

30 “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

Patent Claims

1. An antibody that specifically binds to human ANG2, wherein the antibody comprises a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20, a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; and either a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27, or a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 34; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 35; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 36.
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: 19 and a light chain variable domain that has the amino acid sequence of SEQ ID NO: 6
3. 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: 19 and a light chain variable domain that has the amino acid sequence of SEQ ID NO: 33.
4. 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: 37 and a light chain variable domain that has the amino acid sequence of SEQ ID NO: 42.

5. The antibody according to any one of claims 1 to 4, wherein the antibody is a bispecific antibody.
6. The antibody according to claim 5, wherein the bispecific antibody comprises
 - 5 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,
- 10 wherein either the first antigen or the second antigen is human ANG2.
7. The antibody according to claim 6, wherein the antibody is a bivalent antibody.
8. The antibody according to any one of claims 1 to 7, wherein the antibody is of the human subclass IgG1 or the human subclass IgG4.
- 15 9. The antibody according to claim 8, 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.
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10. The antibody according to claim 8, 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,
5 L368A and Y407V mutations, wherein all numberings are according to the Kabat EU index.
11. A pharmaceutical formulation comprising an antibody according to any one of claims 1 to 10 and optionally a pharmaceutically acceptable carrier.
12. The antibody according to any one of claims 1 to 10 for use as a
10 medicament.
13. Use of the antibody according to any one of claims 1 to 10 in the manufacture of a medicament.