One aspect as reported herein is an antibody comprising a first Fc-region polypeptide and a second Fc-region polypeptide, whereinthe first and a second Fc-region polypeptide are both of human IgG1 or human IgG4 subclass and comprise one or two of the mutations selected from the group I253A, H310A and H435A (numbering according to Kabat EU index numbering system) in the first Fc-region polypeptide and one or two of the mutations selected from the group comprising the mutations I253A, H310A, H435A (numbering according to Kabat EU index numbering system) in the second Fc-region polypeptide so that all of the mutations in the first and the second Fc-region

polypeptide when taken together result in that the mutations I253A, H310A and H435A are comprised in the Fc-region.

There is reported herein the use of an antibody comprising an Fc-region with abolished FcRn binding for the transport of a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.

In one embodiment of all aspects the antibody is not a bispecific, bivalent antibody comprising a first antigen-binding site that specifically binds to human VEGF and a second antigen-binding site that specifically binds to human ANG-2.

Another aspect as reported herein is use of an antibody as reported herein in the manufacture of a medicament for the transport of a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.

Another aspect as reported herein is an antibody as reported herein for use as a medicament.

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

Another aspect as reported herein is use of an antibody as reported herein in the manufacture of a medicament for the removal of one or more soluble receptor ligands from the eye.

Another aspect as reported herein is an antibody as reported herein for use in the removal of one or more soluble receptor ligands from the eye.

Another aspect as reportined herein is use of an antibody as reported herein in the manufacture of a medicament for the treatment of eye diseases, especially of ocular vascular diseases.

Another aspect as reported herein is use of an antibody as reported herein in the manufacture of a medicament for the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.

Another aspect as reported herein is an antibody as reported herein for use in the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.

Another aspect as reported herein is an antibody as reported herein for use in treating an eye disease.

Another aspect as reported herein is an antibody as reported herein for use in the transport of a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.

Another aspect as reported herein is an antibody as reported herein for use in the removal of one or more soluble receptor ligands from the eye.

Another aspect as reported herein is an antibody as reported herein for use in treating eye diseases, especially ocular vascular diseases.

Another aspect as reported herein is an antibody as reported herein for use in the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.

Another aspect as reported herein is an antibody as reported herein for use in transporting a soluble receptor ligand from the intravitreal space or the eye over the blood-ocular-barrier into the blood circulation.

Another aspect as reported herein is use of an antibody as reported herein in the manufacture of a medicament for the transport of a soluble receptor ligand from the intravitreal space or the eye over the blood-ocular-barrier into the blood circulation.

Another aspect as reported herein is a pharmaceutical formulation comprising the antibody as reported herein.

Another aspect as reported herein is a pharmaceutical formulation comprising the antibody as reported herein for use in the treatment of ocular vascular diseases.

Another aspect as reported herein is the use of the antibody as reported herein for the manufacture of a medicament for the treatment of ocular vascular diseases.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Scheme of concept and advantages of <VEGF-ANG-2> IgG1 or IgG4 antibodies with IHH-AAA mutation (= combination of mutations I253A, H310A and H435A (numbering according to EU Index of Kabat)).

Figure 2: Small-scale DLS-based viscosity measurement: Extrapolated viscosity at 150 mg/mL in 200 mM arginine/succinate, pH 5.5 (comparison of <VEGF-ANG-2> antibodies VEGFang2-0016 (with IHH-AAA mutation) with a reference antibody VEGFang2-0015 (without such IHH-AAA mutations)).

Figure 3: DLS Aggregation depending on temperature (including DLS aggregation onset temperature) in 20 mM histidine buffer, 140 mM NaCl, pH 6.0 (comparison of <VEGF-ANG-2> antibodies as reported herein VEGFang2-0016 (with IHH-AAA mutation) with a reference antibody VEGFang2-0015 (without such IHH-AAA mutation)).

Figure 4: Seven day storage at 40 °C at 100 mg/mL (decrease of Main Peak and High Molecular Weight (HMW) increase) (comparison of <VEGF-ANG-2> antibodies as reported herein VEGFang2-0016 (with IHH-AAA mutation) which

showed a lower aggregation with a reference antibody VEGFang2-0015 (without such IHH-AAA mutation)).

Figure 5A and B: FcRn steady state affinity of A: VEGFang2-0015 (without IHH-AAA mutation) and B: VEGFang2-0016 (with IHH-AAA mutation).

Figure 6: FcgammaRIIIa interaction measurement of VEGFang2-0015 without IHH-AAA mutation and VEGFang2-0016 with IHH-AAA mutation (both are IgG1 subclass with P329G LALA mutations; as controls an anti-digoxigenin antibody (anti-Dig) of IgG1 subclass and an IgG4 based antibody were used).

Patent Claims

1. An antibody comprising a first Fc-region polypeptide and a second Fc-region polypeptide, wherein the first and a second Fc-region polypeptide are both of human IgG1 or human IgG4 subclass and comprise one or two of the mutations selected from the group I253A, H310A and H435A

(numbering according to Kabat EU index numbering system) in the first Fc-region polypeptide and one or two of the mutations selected from the group comprising the mutations I253A, H310A, H435A (numbering according to Kabat EU index numbering system) in the second Fc-region polypeptide so that all of the mutations in the first and the second Fc-region polypeptide when taken together result in that the mutations I253A, H310A and H435A are comprised in the Fc-region.

2. The antibody according to claim 1, characterized in that the antibody is a bispecific antibody.

3. The antibody according to claim 1 or 2, characterized in that the antibody is a bivalent antibody.

4. The antibody according to any one of claims 1 to 3, characterized in that

i) the first Fc-region polypeptide is selected from the group comprising
- human IgG1 Fc-region polypeptide,
- human IgG2 Fc-region polypeptide,
- human IgG3 Fc-region polypeptide,
- human IgG4 Fc-region polypeptide,
- human IgG1 Fc-region polypeptide with the mutations L234A, L235A,
- human IgG1 Fc-region polypeptide with the mutations Y349C, T366S, L368A, Y407V,
- human IgG1 Fc-region polypeptide with the mutations S354C, T366S, L368A, Y407V,
- human IgG1 Fc-region polypeptide with the mutations L234A, L235A, Y349C, T366S, L368A, Y407V,
- human IgG1 Fc-region polypeptide with the mutations L234A, L235A, S354C, T366S, L368A, Y407V,

- human IgG1 Fc-region polypeptide with the mutations P329G,
- human IgG1 Fc-region polypeptide with the mutations L234A, L235A, P329G,
- human IgG1 Fc-region polypeptide with the mutations P329G, Y349C, T366S, L368A, Y407V,
- human IgG1 Fc-region polypeptide with the mutations P329G, S354C, T366S, L368A, Y407V,
- human IgG1 Fc-region polypeptide with the mutations L234A, L235A, P329G, Y349C, T366S, L368A, Y407V,
- human IgG1 Fc-region polypeptide with the mutations L234A, L235A, P329G, S354C, T366S, L368A, Y407V,
- human IgG4 Fc-region polypeptide with the mutations S228P, L235E,
- human IgG4 Fc-region polypeptide with the mutations S228P, L235E, P329G,
- human IgG4 Fc-region polypeptide with the mutations Y349C, T366S, L368A, Y407V,
- human IgG4 Fc-region polypeptide with the mutations S354C, T366S, L368A, Y407V,
- human IgG4 Fc-region polypeptide with the mutations S228P, L235E, Y349C, T366S, L368A, Y407V,
- human IgG4 Fc-region polypeptide with the mutations S228P, L235E, S354C, T366S, L368A, Y407V,
- human IgG4 Fc-region polypeptide with the mutations P329G,
- human IgG4 Fc-region polypeptide with the mutations P329G, Y349C, T366S, L368A, Y407V,
- human IgG4 Fc-region polypeptide with the mutations P329G, S354C, T366S, L368A, Y407V,
- human IgG4 Fc-region polypeptide with the mutations S228P, L235E, P329G, Y349C, T366S, L368A, Y407V,
- human IgG4 Fc-region polypeptide with the mutations S228P, L235E, P329G, S354C, T366S, L368A, Y407V,
- human IgG1, IgG2 or IgG4 with the mutations K392D, and
- human IgG3 with the mutation N392D,

and

ii) the second Fc-region polypeptide is selected from the group comprising

- human IgG1 Fc-region polypeptide,
- human IgG2 Fc-region polypeptide,

- human IgG3 Fc-region polypeptide,
- human IgG4 Fc-region polypeptide,
- human IgG1 Fc-region polypeptide with the mutations L234A, L235A,
- human IgG1 Fc-region polypeptide with the mutations S354C, T366W,
- human IgG1 Fc-region polypeptide with the mutations Y349C, T366W,
- human IgG1 Fc-region polypeptide with the mutations L234A, L235A, S354C, T366W,
- human IgG1 Fc-region polypeptide with the mutations L234A, L235A, Y349C, T366W,
- human IgG1 Fc-region polypeptide with the mutations P329G,
- human IgG1 Fc-region polypeptide with the mutations L234A, L235A, P329G,
- human IgG1 Fc-region polypeptide with the mutations P329G, S354C, T366W,
- human IgG1 Fc-region polypeptide with the mutations P329G, Y349C, T366W,
- human IgG1 Fc-region polypeptide with the mutations L234A, L235A, P329G, S354C, T366W,
- human IgG1 Fc-region polypeptide with the mutations L234A, L235A, P329G, Y349C, T366W,
- human IgG4 Fc-region polypeptide with the mutations S228P, L235E,
- human IgG4 Fc-region polypeptide with the mutations S228P, L235E, P329G,
- human IgG4 Fc-region polypeptide with the mutations S354C, T366W,
- human IgG4 Fc-region polypeptide with the mutations Y349C, T366W,
- human IgG4 Fc-region polypeptide with the mutations S228P, L235E, S354C, T366W,
- human IgG4 Fc-region polypeptide with the mutations S228P, L235E, Y349C, T366W,
- human IgG4 Fc-region polypeptide with the mutations P329G,
- human IgG4 Fc-region polypeptide with the mutations P329G, S354C, T366W,

- human IgG4 Fc-region polypeptide with the mutations P329G, Y349C, T366W,
- human IgG4 Fc-region polypeptide with the mutations S228P, L235E, P329G, S354C, T366W,
- human IgG4 Fc-region polypeptide with the mutations S228P, L235E, P329G, Y349C, T366W,
- human IgG1 with the mutations D399K, D356K, and/or E357K, and
- human IgG2, IgG3 or IgG4 with the mutations D399K, E356K, and/or E357K.

5. The antibody according to any one of claims 1 to 3, characterized in that

i) the first Fc-region polypeptide has an amino acid sequence selected from the group comprising SEQ ID NO: 60, 61, 62, 63, 64, 65, 67, 69, 70, 71, 73, 75, 76, 78, 80, 81, 82, and 84, and

ii) the second Fc-region polypeptide has an amino acid sequence selected from the group comprising SEQ ID NO: 60, 61, 62, 63, 64, 66, 68, 69, 70, 72, 74, 75, 76, 77, 79, 81, 83, and 85.

6. The antibody according to any one of claims 1 to 3, characterized in that

i) the first Fc-region polypeptide is a human IgG1 Fc-region polypeptide and the second Fc-region polypeptide is a human IgG1 Fc-region polypeptide, or

ii) the first Fc-region polypeptide is a human IgG1 Fc-region polypeptide with the mutations L234A, L235A and the second Fc-region polypeptide is a human IgG1 Fc-region polypeptide with the mutations L234A, L235A, or

iii) the first Fc-region polypeptide is a human IgG1 Fc-region polypeptide with the mutations L234A, L235A, P329G and the second Fc-region polypeptide is a human IgG1 Fc-region polypeptide with the mutations L234A, L235A, P329G, or

iv) the first Fc-region polypeptide is a human IgG1 Fc-region polypeptide with the mutations L234A, L235A, S354C, T366W and the second Fc-region polypeptide is a human IgG1 Fc-region polypeptide with the mutations L234A, L235A, Y349C, T366S, L368A, Y407V, or

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

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

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

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

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

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.

7. The antibody according to any one of claims 1 to 3, characterized in that

i) the first Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 60 and the second Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 60, or

ii) the first Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 64 and the second Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 64, or

iii) the first Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 70 and the second Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 70, or

iv) the first Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 68 and the second Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 67, or

v) the first Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 74 and the second Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 73, or

vi) the first Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 63 and the second Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 63, or

vii) the first Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 75 and the second Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 75, or

viii) the first Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 76 and the second Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 76, or

ix) the first Fc-region polypeptide is has the amino acid sequence of SEQ ID NO: 79 and the second Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 80, or

x) the first Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 85 and the second Fc-region polypeptide has the amino acid sequence of SEQ ID NO: 84.

8. The antibody according to any one of claims 2 to 7, characterized in that the bispecific antibody has one binding specificity that specifically binds to human ANG-2 and one binding specificity that specifically binds to human VEGF.

9. The antibody according to any one of claims 2 to 8, characterized in that the antibody comprises a first antigen-binding site that specifically binds to

human VEGF and a second antigen-binding site that specifically binds to human ANG-2,

wherein

i) the first antigen-binding site specifically binding to VEGF comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 14, a CDR2H region of SEQ ID NO: 15, and a CDR1H region of SEQ ID NO: 16, and in the light chain variable domain a CDR3L region of SEQ ID NO: 17, a CDR2L region of SEQ ID NO: 18, and a CDR1L region of SEQ ID NO: 19, and

ii) second antigen-binding site specifically binding to ANG-2 comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 22, a CDR2H region of, SEQ ID NO: 23, and a CDR1H region of SEQ ID NO: 24, and in the light chain variable domain a CDR3L region of SEQ ID NO: 25, a CDR2L region of SEQ ID NO: 26, and a CDR1L region of SEQ ID NO: 27.

10. The antibody according to any one of claims 2 to 9, characterized in that the antibody comprises a first antigen-binding site that specifically binds to human VEGF and a second antigen-binding site that specifically binds to human ANG-2,

wherein

i) the first antigen-binding site specifically binding to VEGF comprises as heavy chain variable domain VH an amino acid sequence of SEQ ID NO: 20 and as light chain variable domain VL an amino acid sequence of SEQ ID NO: 21, and

ii) the second antigen-binding site specifically binding to ANG-2 comprises as heavy chain variable domain VH an amino acid sequence of SEQ ID NO: 28 and as light chain variable domain VL an amino acid sequence of SEQ ID NO: 29.

11. The antibody according to any one of claims 2 to 10, characterized in comprising

a) the heavy chain and the light chain of a first full length antibody that specifically binds to VEGF, and

b) the modified heavy chain and modified light chain of a second full length antibody that specifically binds to ANG-2, in which the constant domains CL and CH1 are replaced by each other.

12. The antibody according to any one of claims 2 to 10, characterized in in comprising

    a) the heavy chain and the light chain of a first full length antibody that specifically binds to VEGF, and

    b) the heavy chain and the light chain of a second full length antibody that specifically binds to ANG-2, wherein the N-terminus of the heavy chain is connected to the C-terminus of the light chain via a peptidic linker.

13. The antibody according to claim 12, characterized in that the antibody heavy chain variable domain (VH) and the antibody light chain variable domain (VL) of the heavy and light chain of the second full length antibody are disulfide stabilized by introduction of a disulfide bond between the heavy chain variable domain position 44 and light chain variable domain position 100 (numbering according to Kabat EU index numbering system).

14. An antibody according to any one of claims 1 to 13, characterized in that the antibody does not bind to human FcRn.

15. An antibody according to any one of claims 1 to 14, characterized in that the antibody does bind or does not bind to Staphylococcal protein A.

16. An antibody according to any one of claims 14 to 15, characterized in that the binding or not binding is determined by surface plasmon resonance.

17. An antibody according to any one of claims 1 to 16, characterized in that the antibody

- shows a lower serum concentration compared to corresponding bispecific antibody without the mutations in the Fc-region polypeptides (96 hours after intravitreal application in mice, which are mouse FcRn deficient, but hemizygous transgenic for human FcRn) (determined in assays as described in Example 6), and/or

- shows a similar (factor 0.8 to 1.2) concentration in whole right eye lysates compared to corresponding bispecific antibody without the mutations in the Fc-region polypeptides (in mice, which are mouse FcRn deficient, but hemizygous transgenic for human FcRn, 96 hours after intravitreal application in the right eye) (determined in assays as described in Example 6).

18. Use of an antibody according to any one of claims 1 to 17 in the manufacture of a medicament for the transport of a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.

19. An antibody according to any one of claims 1 to 17 for use as a medicament.

20. An antibody according to any one of claims 1 to 17 for use in the treatment of an ocular vascular disease.

21. Use of an antibody according to any one of claims 1 to 17 in the manufacture of a medicament for the removal of one or more soluble receptor ligands from the eye.

22. An antibody according to any one of claims 1 to 17 for use in the removal of one or more soluble receptor ligands from the eye.

23. Use of an antibody according to any one of claims 1 to 17 in the manufacture of a medicament for the treatment of eye diseases, especially of ocular vascular diseases.

24. Use of an antibody according to any one of claims 1 to 17 in the manufacture of a medicament for the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.

25. An antibody according to any one of claims 1 to 17 for use in the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.

26. An antibody according to any one of claims 1 to 17 for use in treating an eye disease.

27. An antibody according to any one of claims 1 to 17 for use in the transport of a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.

28. An antibody according to any one of claims 1 to 17 for use in the removal of one or more soluble receptor ligands from the eye.

29. An antibody according to any one of claims 1 to 17 for use in treating eye diseases, especially ocular vascular diseases.

30. An antibody according to any one of claims 1 to 17 for use in the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.

31. An antibody according to any one of claims 1 to 17 for use in transporting a soluble receptor ligand from the intravitreal space or the eye over the blood-ocular-barrier into the blood circulation.

32. Use of an antibody according to any one of claims 1 to 17 in the manufacture of a medicament for the transport of a soluble receptor ligand from the intravitreal space or the eye over the blood-ocular-barrier into the blood circulation.

33. A pharmaceutical formulation comprising the antibody according to any one of claims 1 to 17.

34. A pharmaceutical formulation comprising the antibody according to any one of claims 1 to 17 for use in the treatment of ocular vascular diseases.

35. Use of the antibody according to any one of claims 1 to 17 for the manufacture of a medicament for the treatment of ocular vascular diseases.

36. The pharmaceutical formulation according to any one of claims 33 to 34, characterized in that the antibody is to be administered via intravitreal application.

One aspect as reported herein is an antibody comprising a first Fc-region polypeptide and a second Fc-region polypeptide, whereby the antibody comprises the combination of mutations

i) I253A, H310A, and H435A, or

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,

whereby in case of i) the antibody is not a bispecific, bivalent antibody comprising a first antigen-binding site that specifically binds to human VEGF and a second antigen-binding site that specifically binds to human ANG-2,

wherein

the antibody comprises the amino acid sequence of SEQ ID NO: 34, 35, 36 and 37, or

the antibody comprises the amino acid sequence of SEQ ID NO: 38, 39, 40, and 41, or

the antibody comprises the amino acid sequence of SEQ ID NO: 42, 43, 44, and 45, or

the antibody comprises the amino acid sequence of SEQ ID NO: 46, 47, and 48, or

the antibody comprises the amino acid sequence of SEQ ID NO: 49, 50, and 51.

Another aspect as reported herein is an antibody comprising a first Fc-region polypeptide and a second Fc-region polypeptide, whereby the antibody specifically binds to human FcRn with an affinity comparable to or less than an antibody that has the combination of mutations

i) I253A, H310A, and H435A, or

ii) H310A, H433A, and Y436A, or