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 Fab(2) 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-lifes (e.g. due to the faster diffusion into serum) and have to be dosed typically more often.

Kim et al, Molecular Vision, 15 (2009) 2803-2812 relates to full length antibodies administered intravitreally in the eye, wherein an IgG with FcRn binding was eliminated into the blood in wild-type mice, whereas an IgY with no FcRn binding was not eliminated into the blood system. Furthermore the IgG with FcRn binding was not eliminated into the blood system in FcRn knockdown-mice.

There is a need in the art for better means for treating and preventing various ocular vascular diseases such as ischemic retinopathies.

Summary

~~One aspect of t~~The disclosure relates to a ~~is~~ method for the reduction of the viscosity of an antibody wherein the antibody comprises a constant heavy chain region of human IgG1 or human IgG4 subclass (derived from human origin and) wherein the method comprises the modification of the antibody constant heavy chain region of human IgG1 or human IgG4 subclass with the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat).

One aspect of the disclosure is a method for the reduction of the viscosity of an antibody wherein the antibody comprises a constant heavy chain region of human IgG1 or human IgG4 subclass (derived from human origin and) wherein the antibody is a bispecific 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

i) said first antigen-binding site specifically binding to VEGF comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and in the light chain variable domain a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6; and

ii) said second antigen-binding site specifically binding to ANG-2 comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 9, a CDR2H region of, SEQ ID NO: 10, and a CDR1H region of SEQ ID NO: 11, and in the light chain variable domain a CDR3L region of SEQ ID NO: 12, a CDR2L region of SEQ ID NO: 13, and a CDR1L region of SEQ ID NO: 14, and wherein

iii) the bispecific antibody comprises a constant heavy chain region of human IgG1 or human IgG4 subclass (derived from human origin and) comprising the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat)

wherein the method comprises
the modification of the antibody constant heavy chain region of human IgG1 with the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat).

In one embodiment of the disclosure said method is characterized in that the antibody is a bispecific 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

i) said first antigen-binding site specifically binding to VEGF comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and in the light chain variable domain a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6; and

ii) said second antigen-binding site specifically binding to ANG-2 comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 9, a CDR2H region of, SEQ ID NO: 10, and a CDR1H region of SEQ ID NO: 11, and in the light chain variable domain a CDR3L region of SEQ ID NO: 12, a CDR2L region of SEQ ID NO: 13, and a CDR1L region of SEQ ID NO: 14, and wherein

iii) the bispecific antibody comprises a constant heavy chain region of human IgG1 or human IgG4 subclass (derived from human origin and) comprising the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat).

In one embodiment of the disclosure such method is characterized in that said bispecific antibody described above comprises a constant heavy chain region of human IgG1 subclass (derived from human origin and) comprising the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat) and further comprising the mutations L234A , L235A and P329G (numbering according to EU Index of Kabat).

One ~~aspect~~ embodiment of the disclosure is an antibody obtained by such method.

~~One aspect of t~~The disclosure also relates to ~~is~~ the use of the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat) for the reduction of the viscosity of an antibody wherein the antibody comprises a constant heavy chain region of human IgG1 or human IgG4 subclass (derived from human origin).

One aspect of the disclosure is the use of the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat) for the reduction of the viscosity of an antibody wherein the antibody comprises a constant heavy chain region of human IgG1 or human IgG4 subclass(derived from human origin); and wherein the antibody is a bispecific 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

i) said first antigen-binding site specifically binding to VEGF comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and in the light chain variable domain a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6; and

ii) said second antigen-binding site specifically binding to ANG-2 comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 9, a CDR2H region of, SEQ ID NO: 10, and a CDR1H region of SEQ ID NO: 11, and in the light chain variable domain a CDR3L region of SEQ ID NO: 12, a CDR2L region of SEQ ID NO: 13, and a CDR1L region of SEQ ID NO: 14, and wherein

iii) the bispecific antibody comprises a constant heavy chain region of human IgG1 (derived from human origin and) comprising the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat).

In one embodiment of the disclosure said use is characterized in that the antibody is a bispecific 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

i) said first antigen-binding site specifically binding to VEGF comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and in the light chain variable domain a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6; and

a second antigen-binding site that specifically binds to human ANG-2, characterized in comprising the amino acid sequences of SEQ ID NO: 25, of SEQ ID NO: 26, of SEQ ID NO: 27, and of SEQ ID NO: 28.

~~Accordingly one aspect of the disclosure is 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, characterized in comprising the amino acid sequences of SEQ ID NO: 29, of SEQ ID NO: 30, of SEQ ID NO: 31, and of SEQ ID NO: 32.~~

One aspect of the disclosure ~~if~~ is the use of the bispecific antibody as defined herein ~~for~~ in the manufacture of a medicament for the treatment of ocular vascular diseases.

The antibodies according to the disclosure have highly valuable properties due to their specific modifications in the Fc part/ constant region causing a benefit for a patient suffering from ocular vascular diseases. They show high stability in the intravitreal environment and slow diffusion from the eye (compared to smaller antibody fragments without a constant heavy chain region), where the actual disease is located and treated (so treatment schedule can potentially be improved compared to non-IgG like antibodies like e.g. Fab and (Fab)2 fragments). Surprisingly compared to unmodified IgG antibodies the half-life in the eye after intravitreal application of the antibodies with the mutations I253A, H310A, and H435A in the constant region (with no more FcRn binding) was similar (only slightly reduced) (Tables 17a and 18a and Figures 7D and 7E), whereas the diffusion from the eye into the blood serum was similar (Table 15 and Fig7B). This highly valuable as it is desired for the treatment of ocular vascular diseases related to ANG2 and/or VEGF it to eliminate VEGF und Ang2 from the eye (e. via the transportation into the blood serum as anti-ANG2/ANG2 antibody complex or anti-VEGF/VEGF antibody complex). The antibodies according to the disclosure are cleared on the other hand quite rapidly from serum when compared to unmodified IgG antibodies (which is highly desired to reduce potential side effects arising from systemic exposure).

Surprisingly they also show lower viscosity (see Figure 2) (compared to versions without the mutations I253A, H310A, and H435A in the constant region) and are therefore especially useful for intravitreal application through thin needles during the treatment of eye diseases (for such application typically thin needles are used and high viscosity makes an appropriate application rather difficult). The lower viscosity also allows higher concentration formulations.

Patent Claims

1. A method for the reduction of the viscosity of an antibody wherein the antibody comprises a constant heavy chain region of human IgG1 or human IgG4 subclass (derived from human origin and) wherein the antibody is a bispecific 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

i) said first antigen-binding site specifically binding to VEGF comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and in the light chain variable domain a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6; and

ii) said second antigen-binding site specifically binding to ANG-2 comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 9, a CDR2H region of, SEQ ID NO: 10, and a CDR1H region of SEQ ID NO: 11, and in the light chain variable domain a CDR3L region of SEQ ID NO: 12, a CDR2L region of SEQ ID NO: 13, and a CDR1L region of SEQ ID NO: 14, and wherein

iii) the bispecific antibody comprises a constant heavy chain region of human IgG1 or human IgG4 subclass (derived from human origin and) comprising the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat)

wherein the method comprises
the modification of the antibody constant heavy chain region of human IgG1 ~~or human IgG4 subclass~~ with the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat).

~~2. The method of claim 1, wherein the antibody is a bispecific 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~~

~~i) said first antigen-binding site specifically binding to VEGF comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and in the light chain variable domain a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6; and~~

~~ii) said second antigen-binding site specifically binding to ANG-2 comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 9, a CDR2H region of, SEQ ID NO: 10, and a CDR1H region of SEQ ID NO: 11, and in the light chain variable domain a CDR3L region of SEQ ID NO: 12, a CDR2L region of SEQ ID NO: 13, and a CDR1L region of SEQ ID NO: 14, and wherein~~

~~iii) the bispecific antibody comprises a constant heavy chain region of human IgG1 or human IgG4 subclass (derived from human origin and) comprising the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat).~~

~~3.~~2. The method of claim ~~2~~1, wherein the bispecific antibody comprises a constant heavy chain region of human IgG1 subclass (derived from human origin and) comprising the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat) and further comprising the mutations L234A , L235A and P329G (numbering according to EU Index of Kabat).

~~4. An antibody obtained by the method of any one of claims 1 to 3.~~

~~5.~~3. Use of the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat) for the reduction of the viscosity of an antibody wherein the antibody comprises a constant heavy chain region of human IgG1 or human IgG4 subclass(derived from human origin)~~.~~; and

~~6. The use of claim 5,~~ wherein the antibody is a bispecific 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

i) said first antigen-binding site specifically binding to VEGF comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and in the light chain variable domain a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6; and

ii) said second antigen-binding site specifically binding to ANG-2 comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 9, a CDR2H region of, SEQ ID NO: 10, and a CDR1H region of SEQ ID NO: 11, and in the light chain variable domain a CDR3L region of SEQ ID NO: 12, a CDR2L region of SEQ ID NO: 13, and a CDR1L region of SEQ ID NO: 14, and wherein

iii) the bispecific antibody comprises a constant heavy chain region of human IgG1 ~~or human IgG4 subclass~~ (derived from human origin and) comprising the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat).

~~7.~~4. The use of claim ~~6~~3, wherein the bispecific antibody comprises a constant heavy chain region of human IgG1 subclass (derived from human origin and) comprising the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat) and further comprising the mutations L234A , L235A and P329G (numbering according to EU Index of Kabat).

~~8.~~5. A bispecific 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

i) said first antigen-binding site specifically binding to VEGF comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and in the light chain variable domain a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6; and

ii) said second antigen-binding site specifically binding to ANG-2 comprises in the heavy chain variable domain a CDR3H region of SEQ ID NO: 9, a CDR2H region of, SEQ ID NO: 10, and a CDR1H region of SEQ ID NO: 11, and in the light chain variable domain a CDR3L region of SEQ ID NO: 12, a CDR2L region of SEQ ID NO: 13, and a CDR1L region of SEQ ID NO: 14, and wherein

iii) the bispecific antibody comprises a constant heavy chain region of human IgG1 ~~or human IgG4 subclass~~ (derived from human origin and) comprising the mutations I253A, H310A, and H435A (numbering according to EU Index of Kabat)

~~9~~6. The bispecific antibody according to claim ~~8~~5, wherein

i) said first antigen-binding site specifically binding to VEGF comprises as heavy chain variable domain VH an amino acid sequence of SEQ ID NO: 7, and as light chain variable domain VL an amino acid sequence of SEQ ID NO: 8, and

ii) said second antigen-binding site specifically binding to ANG-2 comprises as heavy chain variable domain VH an amino acid sequence of SEQ ID NO: 15, and as light chain variable domain VL an amino acid sequence of SEQ ID NO: 16.

~~10~~7. The bispecific antibody according to any one of claims ~~8~~5 to ~~9~~6, wherein the constant heavy chain region under iii) is of IgG1 subclass.

~~11~~8. The bispecific antibody according to claim ~~10~~7, wherein the constant heavy chain region of IgG1 subclass further comprises the mutations L234A , L235A and P329G (numbering according to EU Index of Kabat)

~~12~~9. The bispecific antibody according to any one of claims ~~8~~5 to ~~9~~6, wherein the constant heavy chain region under iii) is of IgG4 subclass

~~13. The bispecific antibody according to claim 12, wherein the constant heavy chain region of IgG4 subclass further comprises the mutations S228P and L235E (numbering according to EU Index of Kabat)~~

~~14. The bispecific antibody according to claim 12, wherein the constant heavy chain region of IgG4 subclass further comprises the mutations S228P , L235E and P329G (numbering according to EU Index of Kabat)~~

~~15~~10. A pharmaceutical composition comprising an antibody according to any one of claims ~~4 and 8~~5 to ~~14~~9.

~~16~~11. The bispecific antibody according to any one of claims ~~4 and 8~~5 to ~~14~~9 for use in the treatment of ocular vascular diseases.

~~17~~12. The bispecific antibody according to claim ~~16~~11, wherein the antibody is administered via intravitreal application.

~~18~~13. A nucleic acid encoding a bispecific antibody according to any one of claims ~~8~~5 to ~~14~~9.

~~19~~14. Expression vector containing said nucleic acid according claim ~~18~~13 capable of expressing said nucleic acid in a prokaryotic or eukaryotic host cell.

~~20~~15. A prokaryotic or eukaryotic host cell comprising a vector according to claim ~~19~~14.

~~21~~16. A method for the preparation of a bispecific antibody according to any one of claims ~~8~~5 to ~~14~~9

comprising the steps of

a) transforming a host cell with vectors comprising nucleic acid molecules encoding said antibody;

b) culturing the host cell under conditions that allow synthesis of said antibody molecule; and

c) recovering said antibody molecule from said culture.

~~22~~17. A bispecific antibody obtained by the method of claim ~~21~~16.

~~23~~18. 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, characterized in comprising the amino acid sequences of SEQ ID NO: 25, of SEQ ID NO: 26, of SEQ ID NO: 27, and of SEQ ID NO: 28.

~~24~~19. 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, characterized in comprising the amino acid sequences of SEQ ID NO: 21, of SEQ ID NO: 22., of SEQ ID NO: 23., and of SEQ ID NO: 24.

~~25. 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, characterized in comprising the amino acid sequences of SEQ ID NO: 29, of SEQ ID NO: 30, of SEQ ID NO: 31, and of SEQ ID NO: 32.~~

~~26~~20. The bispecific antibody according to any one of claims claims ~~23~~18 to ~~25~~19 ~~for~~ for use in the treatment of ocular vascular diseases.

~~27~~21. The bispecific antibody according to claim ~~26~~20, wherein the antibody is administered via intravitreal application.

~~28~~22. Use of the bispecific antibody according to any one of claims ~~4 and 8~~ 5 to ~~14~~9, and claims ~~23~~18 to ~~25~~19 ~~for~~ in the manufacture of a medicament for the treatment of ocular vascular diseases.

~~29~~23. Use of claim ~~28~~22, wherein the antibody is administered via intravitreal application.

~~30. A method of treatment of patient suffering from ocular vascular diseases by administering an antibody according to any one of claims 4 and 8 to 14, and claims 23 to 25 to a patient in the need of such treatment.~~

~~31. The method of claim 30, wherein the antibody is administered via intravitreal application.~~