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SUMMARY

For using an antibody that targets/binds to antigens not only present in the eye but also in the remaining body a short systemic half-live after passage of the blood-ocular-barrier from the eye into the blood is beneficial in order to avoid side effects.

Also for an antibody that specifically binds to a ligand of a receptor and that is effective in the treatment of eye diseases the antibody-antigen complex (=antibody-receptor ligand-complex) has after the removal from the eye and thereafter the antibody (either after dissociation or as complex) has to be removed from the systemic circulation in order to avoid side effects, i.e. the antibody functions as a vehicle for removal of receptor ligands from the eye, thereby inhibits receptor signaling and is cleared fast from the blood circulation in order to avoid systemic side effects.

It has been found that an antibody comprising an Fc-region that does not bind to the human neonatal Fc-receptor can pass the blood-ocular-barrier. This is surprising as the antibody does not bind to human FcRn although binding to FcRn is considered to be required for transport across the blood-ocular-barrier.

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

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, wherein

i) the first and the second Fc-region polypeptide comprise the mutations I253A, H310A and H435A, or

ii) the first and the second Fc-region polypeptide comprise the mutations H310A, H433A and Y436A, or

iii) the first and the second Fc-region polypeptide comprise the mutations L251D, L314D and L432D, or

iv) the first Fc-region polypeptide comprises the mutations I253A, H310A and H435A and the second Fc-region polypeptide comprises

a) the mutations H310A, H433A and Y436A, or

b) the mutations L251D, L314D and L432D,

or

v) the first Fc-region polypeptide comprises the mutations H310A, H433A and Y436A and the second Fc-region polypeptide comprises

a) the mutations L251D, L314D and L432D.

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, wherein

α) the first and a second Fc-region polypeptide are both of human IgG1 or human IgG4 subclass (derived from human origin) and comprise one or two of the mutations selected from i) the group I253A, H310A and H435A, or ii) the group H310A, H433A and Y436A, or iii) the group L251D, L314D and L432D (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 L251D, I253A, H310A, L314D, L432D, H433A, H435A and Y436A (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 i) I253A, H310A and H435A, or ii) H310A, H433A and Y436A, or iii) L251D, L314D and L432D are comprised in the Fc-region, or

β) the first and a second Fc-region polypeptide both of human IgG1 or human IgG4 subclass (i.e. derived from human origin) and both comprise the mutations I253A/H310A/H435A or H310A/H433A/Y436A or L251D/L314D/L432D or combinations thereof in the Fc-region (numbering according to Kabat EU index numbering system), whereby either all mutations are in the first or the second Fc-region polypeptide, or one or two mutations are in the first Fc-region polypeptide and one or two mutations are 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 i) I253A, H310A and H435A, or ii) H310A, H433A and Y436A, or iii) L251D, L314D and L432D are comprised in the Fc-region, or

γ) the first and a second Fc-region polypeptide both of human IgG1 or human IgG4 subclass (i.e. derived from human origin) and comprise the mutations I253A/H310A/H435A or H310A/H433A/Y436A or L251D/L314D/L432D in the first as well as in the second Fc-region polypeptide (numbering according to Kabat EU index numbering system), or comprises the combinations of the mutations I253A/H310A/H435A in the first Fc-region polypeptide and the combination of the mutations H310A/H433A/Y436A in the second Fc-region polypeptide (numbering according to Kabat EU index numbering system),

whereby in case of the combination of the mutations I253A/H310A/H435A in both Fc-region polypeptides 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.

~~One aspect~~ There is ~~as~~ reported herein ~~is~~ 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.

There is ~~One aspect as~~ reported herein ~~is~~ an antibody comprising an Fc-region with abolished FcRn binding for use as a medicament.

There is ~~One aspect as~~ reported herein ~~is~~ an antibody comprising an Fc-region with abolished FcRn binding for use in the treatment of an ocular vascular disease.

There is ~~One aspect as~~ reported herein ~~is~~ the use of an antibody comprising an Fc-region with abolished FcRn binding for the removal of one or more soluble receptor ligands from the eye.

There is ~~One aspect as~~ reported herein ~~is~~ an antibody comprising an Fc-region with abolished FcRn binding for use in the removal of one or more soluble receptor ligands from the eye.

There is ~~One aspect as~~ reported herein ~~is~~ the use of an antibody comprising an Fc-region with abolished FcRn binding for the treatment of eye diseases, especially of ocular vascular diseases.

There is ~~One aspect as~~ reported herein ~~is~~ the use of an antibody comprising an Fc-region with abolished FcRn binding for the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.

There is ~~One aspect as~~ reported herein ~~is~~ an antibody comprising an Fc-region with abolished FcRn binding for use in the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.

In one embodiment of all aspects the antibody has no detectable FcRn binding using surface plasmon resonance.

In one embodiment of all aspects the antibody binds to human FcRn with a Kd value of more than 1.7 μM at pH 6, i.e. with low affinity.

In one embodiment of all aspects the antibody has the same or a shorter retention time on an FcRn affinity chromatography column as an antibody comprising an Fc-Region with the mutations I253A/H310A/H435A or H310A/H433A/Y436A or L251D/L314D/L432D or combinations thereof (numbering according to Kabat EU index numbering system).

In one embodiment of all aspects the antibody has a retention time on an FcRn affinity chromatography column of three minutes or less.

In one embodiment the antibody has at least one of the mutations L251D, L251S, M252T, I253A, S254W, S254R, H310A, H433A, N434L, H435A, Y436A (numbering according to Kabat EU index numbering system) in the first Fc-region polypeptide and at least one of the mutations L251D, L251S, M252T, I253A, S254W, S254R, H310A, H433A, N434L, H435A, Y436A (numbering according to Kabat EU index numbering system) in the second Fc-region polypeptide.

In one embodiment the antibody has at least the mutations I253A/H310A/H435A or H310A/H433A/Y436A or L251D/L314D/L432D or combinations thereof (numbering according to Kabat EU index numbering system) in both Fc-region polypeptides.

In one embodiment of all aspects the antibody has a heterodimeric Fc-region.

In one embodiment the antibody is a full-length antibody.

In one embodiment the antibody is a monoclonal antibody.

In one embodiment the antibody is a human, humanized or chimeric antibody.

In one embodiment the antibody is a monospecific antibody.

In one embodiment the antibody is a bispecific antibody.

There is ~~One aspect as~~ reported herein ~~is~~ an antibody comprising an Fc-region with abolished FcRn binding for use in treating an eye disease.

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

There is ~~One aspect as~~ reported herein ~~is~~ an antibody comprising an Fc-region with abolished FcRn binding for use in the removal of one or more soluble receptor ligands from the eye.

There is ~~One aspect as~~ reported herein ~~is~~ an antibody comprising an Fc-region with abolished FcRn binding for use in treating eye diseases, especially ocular vascular diseases.

There is ~~One aspect as~~ reported herein ~~is~~ an antibody comprising an Fc-region with abolished FcRn binding for use in the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.

In one embodiment of all aspects the treatment or application of the antibody is by intravitreal application.

In one embodiment of all aspects the antibody has no detectable FcRn binding using surface plasmon resonance.

In one embodiment of all aspects the antibody binds to human FcRn with a Kd value of more than 1.7 µM at pH 6, i.e. with low affinity.

In one embodiment of all aspects the antibody has the same or a shorter retention time on an FcRn affinity chromatography column as an antibody comprising an Fc-region with the mutations I253A/H310A/H435A or H310A/H433A/Y436A or L251D/L314D/L432D or combinations thereof (numbering according to Kabat EU index numbering system).

In one embodiment of all aspects the antibody has a retention time on an FcRn affinity chromatography column of three minutes or less.

In one embodiment the FcRn affinity column has the column dimensions of 50 mm x 5 mm, the bed height is 5 cm and the loading is 50 µg antibody. In one embodiment the equilibration buffer is 20 mM MES, with 150 mM NaCl, adjusted to pH 5.5, the elution buffer is 20 mM Tris/HCl, with 150 mM NaCl, adjusted to pH 8.8 and the elution is by applying 7.5 CV equilibration buffer, from 0 % to 100 % elution buffer in 30 CV, and thereafter 10 CV elution buffer.

In one embodiment of all aspects the antibody binds to human FcRn with about the same or lesser affinity than an antibody comprising an Fc-Region with the mutations I253A/H310A/H435A or H310A/H433A/Y436A or L251D/L314D/L432D or combinations thereof (numbering according to Kabat EU index numbering system).

In one embodiment of all aspects the antibody binds to human FcRn with about the same or lesser affinity than an antibody with a heavy chain amino acid sequence of SEQ ID NO: 01 or SEQ ID NO: 02 or SEQ ID NO: 112 and a light chain amino acid sequence of SEQ ID NO: 03.

In one embodiment the antibody is a human, humanized or chimeric antibody.

In one embodiment the antibody is a monospecific antibody.

In one embodiment the antibody is a bispecific antibody.

There is ~~One aspect as~~ reported herein ~~is~~ a method of treating an individual having an ocular vascular disease comprising administering to the individual an effective amount of an antibody as reported herein.

There is ~~One aspect as~~ reported herein ~~is~~ a method for transporting a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation in an individual comprising administering to the individual an effective amount of an antibody as reported herein to transport a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.

There is ~~One aspect as~~ reported herein ~~is~~ a method for the removal of one or more soluble receptor ligands from the eye in an individual comprising administering to the individual an effective amount of an antibody as reported herein to remove one or more soluble receptor ligands from the eye.

There is ~~One aspect as~~ reported herein ~~is~~ a method for the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation in an individual comprising administering to the individual an effective amount of an antibody as reported herein to transport one or more soluble receptor ligands from the intravitreal space to the blood circulation.

There is ~~One aspect as~~ reported herein ~~is~~ a method for transporting a soluble receptor ligand from the intravitreal space or the eye over the blood-ocular-barrier into the blood circulation in an individual comprising administering to the individual an effective amount of an antibody as reported herein to transport a soluble receptor ligand from the intravitreal space or the eye over the blood-ocular-barrier into the blood circulation.

In one embodiment of all aspects the antibody comprises a first Fc-region polypeptide and a second Fc-region polypeptide wherein

i) the first and the second Fc-region polypeptide comprise the mutation Y436A, or

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 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: 102, of SEQ ID NO: 103, of SEQ ID NO: 36, and of SEQ ID NO: 37.

Another aspect as reported herein 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: 104, of SEQ ID NO: 105, of SEQ ID NO: 40, and of SEQ ID NO: 41.

Another aspect as reported herein 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:106, of SEQ ID NO: 107, of SEQ ID NO: 44, and of SEQ ID NO: 45.

Another aspect as reported herein 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: 108, of SEQ ID NO: 109, and of SEQ ID NO: 48.

Another aspect as reported herein 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: 110, of SEQ ID NO: 111, and of SEQ ID NO: 51.

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 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 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 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).