**(12) STANDARD PATENT** (11) Application No. **AU 2013288641 B2**
**(19) AUSTRALIAN PATENT OFFICE**

(54) Title
**Bispecific anti-VEGF/anti-ANG-2 antibodies and their use in the treatment of ocular vascular diseases**

(51) International Patent Classification(s)
***C07K 16/22*** (2006.01) ***C07K 16/46*** (2006.01)
***C07K 16/28*** (2006.01)

(21) Application No: **2013288641** (22) Date of Filing: **2013.07.11**

(87) WIPO No: **WO14/009465**

(30) Priority Data

| (31) Number | (32) Date | (33) Country |
|---|---|---|
| **12176299.1** | **2012.07.13** | **EP** |

(43) Publication Date: **2014.01.16**
(44) Accepted Journal Date: **2017.07.06**

(71) Applicant(s)
**Roche Glycart AG**

(72) Inventor(s)
**Duerr, Harald;Herting, Frank;Klein, Christian;Regula, Joerg Thomas;Rueth, Matthias;Stubenrauch, Kay-Gunnar**

(74) Agent / Attorney
**Spruson & Ferguson, L 35 St Martins Tower 31 Market St, Sydney, NSW, 2000, AU**

(56) Related Art
**US 6277375 B1**
**KIM J-K ET AL, "Mapping the site on human IgG for binding of the MHC class I-related receptor, FcRn", EUROPEAN JOURNAL OF IMMUNOLOGY, WILEY - V C H VERLAG GMBH & CO. KGAA, DE, (19990901), vol. 29, no. 9, pages 2819 - 2825**

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) **World Intellectual Property Organization**
International Bureau

(43) **International Publication Date**
**16 January 2014 (16.01.2014)**

WIPO | PCT

(10) **International Publication Number**
**WO 2014/009465 A1**

(51) **International Patent Classification**:
***C07K 16/22*** (2006.01) ***C07K 16/28*** (2006.01)
***C07K 16/46*** (2006.01)

(21) **International Application Number**:
PCT/EP2013/064672

(22) **International Filing Date**:
11 July 2013 (11.07.2013)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
12176299.1 13 July 2012 (13.07.2012) EP

(71) **Applicant**: **ROCHE GLYCART AG** [CH/CH]; Wagistrasse 18, CH-8952 Schlieren (CH).

(72) **Inventors**: **DUERR, Harald**; Gustav-Meyrink-Strasse 7a, 82319 Starnberg (DE). **HERTING, Frank**; An der Freiheit 29a, 82377 Penzberg (DE). **KLEIN, Christian**; Chruezacherweg 41, CH-8906 Bonstetten (CH). **REGULA, Joerg**; Lierstrasse 15, 80639 Muenchen (DE). **RUETH, Matthias**; Gabriele-Muenter-Weg 7, 82377 Penzberg (DE). **STUBENRAUCH, Kay-Gunnar**; Parkstrasse 1a, 82377 Penzberg (DE).

(74) **Agents**: **JENNI, Wolfgang** et al.; Roche Diagnostics GmbH, Patent Department (LPP.....6164), P.O.Box 11 52, 82372 Penzberg (DE).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

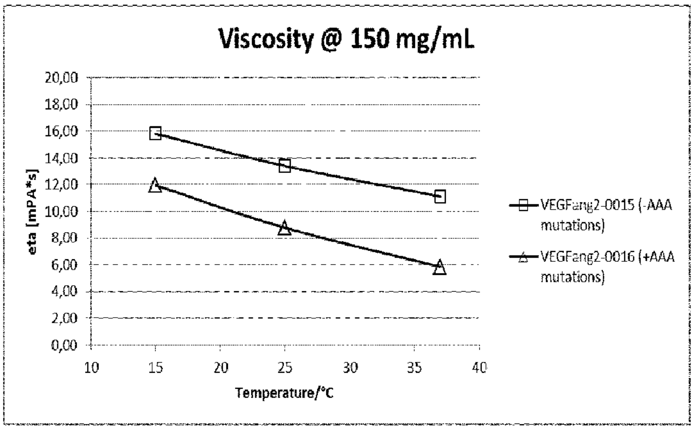
(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Published**:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

# (54) Title: BISPECIFIC ANTI-VEGF/ANTI-ANG-2 ANTIBODIES AND THEIR USE IN THE TREATMENT OF OCULAR VASCULAR DISEASES

Fig. 2



(57) **Abstract**: The present invention relates to bispecific antibody against human vascular endothelial growth factor (VEGF/VEGF-A) and against human angiopoietin-2 (ANG-2) of human IgG1 or IgG4 subclass with mutations I253A, H310A, and H435A, methods for their production, pharmaceutical compositions containing said antibodies, and uses thereof.

WO 2014/009465 A1

# Bispecific anti-VEGF/anti-ANG-2 antibodies and their use in the treatment of ocular vascular diseases

The present invention relates to a method for the reduction of the viscosity of an antibody (including a bispecific antibody) of human IgG1 or human IgG4 subclass, to bispecific antibodies against human vascular endothelial growth factor (VEGF/VEGF-A) and against human angiopoietin-2 (ANG-2), methods for their production, pharmaceutical compositions containing said antibodies, and uses thereof.

## Background of the Invention

Angiogenesis is implicated in the pathogenesis of a variety of disorders which include solid tumors, intraocular neovascular syndromes such as proliferative retinopathies or age-related macular degeneration (AMD), rheumatoid arthritis, and psoriasis (Folkman, J., et al., J. Biol. Chem. 267 (1992) 10931-10934; Klagsbrun, M., et al., Annu. Rev. Physiol. 53 (1991) 217-239; and Garner, A., Vascular diseases, in: Pathobiology of ocular disease, A dynamic approach, Garner, A., and Klintworth, G. K. (eds.), 2nd edition, Marcel Dekker, New York (1994), pp. 1625-1710).

Ranibizumab (trade name Lucentis®) is a monoclonal antibody fragment derived from the same parent murine antibody as bevacizumab (Avastin). However, it has been affinity matured to provide stronger binding to VEGF-A (WO 98/45331). It is known that VEGF-A blocking may be related to some systemic toxicities, therefore ranibizumab is missing an Fc part to reduce the serum half live and consequently systemic toxicities. It is an anti-angiogenic agent that has been approved to treat the "wet" type of age-related macular degeneration (ARMD), a common form of age-related vision loss.

Corneal angiogenesis assays have shown that both ANG-1 and ANG-2 had similar effects, acting synergistically with VEGF to promote growth of new blood vessels. Asahara, T., et al., Circ. Res. 83 (1998) 233-40. The possibility that there was a dose-dependent endothelial response was raised by the observation that in vitro at high concentration, ANG-2 can also be pro-angiogenic (Kim, I., et al., Oncogene 19 (2000) 4549-52). At high concentration, ANG-2 acts as an apoptosis survival factor for endothelial cells during serum deprivation apoptosis through activation of Tie2 via PI-3 Kinase and Akt pathway (Kim, I., et al., Oncogene 19 (2000) 4549-52).

WO 2010/040508 A9 and WO 2011/117329 relate to bispecific anti-VEGF/anti-ANG-2 antibodies. WO 2008/132568 relates to fusion proteins binding to growth factors. WO 2009/136352 relates to anti-angiogenic compounds. WO 2009/080253 and WO 2011/117330 relates to bispecific bivalent antibody formats. WO 2010/069532 relates to Ang2 antibodies.

Ocular vascular diseases such as age related macular degeneration (ARMD) and diabetic retinopathy (DR) are due to abnormal choroidal or retinal neovascularization respectively. They are the leading causes of visual loss in industrialized nations. Since the retina consists of well-defined layers of neuronal, glial, and vascular elements, relatively small disturbances such as those seen in vascular proliferation or edema can lead to significant loss of visual function. Inherited retinal degenerations, such as Retinitis Pigmentosa (RP), are also associated with vascular abnormalities, such as arteriolar narrowing and vascular atrophy. They affect as many as 1 in 3500 individuals and are characterized by progressive night blindness, visual field loss, optic nerve atrophy, arteriolar attenuation, and central loss of vision often progressing to complete blindness.

Ischemic retinopathies are characterized by loss or dysfunction of the retinal vasculature which results in a reduction of blood flow and hypoxia. The retina responds to hypoxia by generating signals to grow new blood vessels, but these new vessels are usually fragile and disorganized. It is the growth of these abnormal new vessels that creates most of the threat to vision since they can leak, hemorrhage or lead to scarring that may end in retinal detachment. Current treatments for ischemic retinopathies seek to halt the growth of the pathological vessels but do not address the underlying ischemia that drives their growth. Furthermore, standard treatment for diabetic retinopathy, an ischemic retinopathy that affects millions, involves destruction of a portion of the retina with a laser in an attempt to stop new vessel growth and preserve central vision. Strategies have been employed to block the function of vascular endothelial growth factor (VEGF), a major promoter of vessel growth. In the short term, anti-VEGF therapy can improve vision, but it does not address the underlying ischemia and in fact may exacerbate this condition as it inhibits all vessel growth, including beneficial collaterals. There is also the serious concern of systemic exposure of these drugs in 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 of the Invention

One aspect of the invention 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).

In one embodiment of the invention 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 invention 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 embodiment of the invention is an antibody obtained by such method.

One embodiment of the invention 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).

In one embodiment of the invention 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

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 invention said specific use is characterized in that 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).

The invention is further directed to 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

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 said bispecific antibody is characterized in that

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.

In one embodiment said bispecific antibody is characterized in that the constant heavy chain region under iii) is of human IgG1 subclass. In one embodiment said bispecific antibody of IgG1 subclass is characterized in that the constant heavy chain region of IgG1 subclass further comprises the mutations L234A , L235A and P329G (numbering according to EU Index of Kabat)

In one embodiment said bispecific antibody is characterized in that the constant heavy chain region under iii) is of human IgG4 subclass. In one embodiment said bispecific antibody of IgG4 subclass is characterized in that the constant heavy chain region of IgG4 subclass further comprises the mutations S228P and L235E (numbering according to EU Index of Kabat). In one embodiment said bispecific antibody of IgG4 subclass is characterized in that the constant heavy chain region of IgG4 subclass further comprises the mutations S228P , L235E and P329G (numbering according to EU Index of Kabat)

Still further aspects of the invention are a pharmaceutical composition comprising said bispecific antibody, said pharmaceutical composition for use in the treatment of ocular vascular diseases, the use of said bispecific antibody for the manufacture of a medicament for the treatment of ocular vascular diseases, a method of treatment of patient suffering from ocular vascular diseases by administering said bispecific antibody to a patient in the need of such treatment. In one embodiment the bispecific antibody or the pharmaceutical composition comprising said bispecific antibody is administered via intravitreal application.

A further aspect of the invention is a nucleic acid molecule encoding a heavy and/or light chain of a bispecific antibody according to the invention.

The invention further provides expression vectors containing said nucleic acid according to the invention capable of expressing said nucleic acid in a prokaryotic or eukaryotic host cell, and host cells containing such vectors for the recombinant production of a bispecific antibody according to the invention.

The invention further comprises a prokaryotic or eukaryotic host cell comprising a vector according to the invention.

The invention further comprises a method for the production of a bispecific antibody according to the invention, characterized by expressing a nucleic acid according to the invention in a prokaryotic or eukaryotic host cell and recovering said bispecific antibody from said cell or the cell culture supernatant. One embodiment is a method for the preparation of a bispecific antibody according to the invention 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

The invention further comprises the antibody obtained by such method for the production of a bispecific antibody.

Accordingly one embodiment of the invention 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: 21, of SEQ ID NO: 22, of SEQ ID NO: 23, and of SEQ ID NO: 24.

Accordingly one embodiment of the invention 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: 25, of SEQ ID NO: 26, of SEQ ID NO: 27, and of SEQ ID NO: 28.

The antibodies according to the invention 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 invention 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.

Also surprisingly the antibodies according to the invention show a lower aggregation tendency (Fig 4) during storage (compared to versions without the mutations I253A, H310A, and H435A in the Fc part) which is critical for intravitreal application in the eye (as an aggregation in the eye can lead to complications during such treatment).The bispecific antibodies according to the invention show good efficacy in inhibition of vascular diseases.

In certain embodiments, the bispecific antibodies according to the invention due to their specific modifications in the constant region (e.g. P329G LALA) show valuable properties like no binding Fcgamma receptors which reduces the risk of side effects like thrombosis and/or unwanted cell death (due to e.g. ADCC)

## Description of the Figures

| | |
|---|---|
| **Figure 1** | Scheme of concept and advantages of <VEGF-ANG-2> IgG1 or IgG4 antibodies with AAA mutations (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 according to the invention VEGFang2-0016 (with AAA mutations) with a reference VEGFang2-0015 (without such AAA mutations) |
| **Figure 3** | DLS Aggregation depending on temperature ( including DLS aggregation onset temperature) in 20 mM His, 140 mM NaCl, pH 6.0 5 (comparison of <VEGF-ANG-2> antibodies according to the invention VEGFang2-0016 (with AAA mutations) with a reference VEGFang2-0015 (without such AAA mutations) |
| **Figure 4** | 7 day storage at 40°C at 100 mg/ml (Decrease of Main and High Molecular Weight /HMW) increase) (comparison of <VEGF-ANG-2> antibodies according to the invention VEGFang2-0016 (with AAA mutations) which showed a lower aggregation with a reference VEGFang2-0015 (without such AAA mutations)) |
| **Figure 5A** | FcRn steady state affinity of VEGFang2-0015 (without AAA mutations): overlay of Biacore sensogramms at different concentrations shows a concentration dependent binding of VEGFang2-0015 (without AAA mutations) to FcRn |
| **Figure 5B** | FcRn steady state affinity of A: VEGFang2-0015 (without AAA mutations): the concentration dependent binding response curve of VEGFang2-0015 (without AAA mutations) shows binding to FcRn |

| | |
|---|---|
| **Figure 5C** | FcRn steady state affinity of VEGFang2-0016 (with AAA mutations): overlay of Biacore sensogramms at different concentrations shows no binding to FcRn at all concentrations |
| **Figure 5D** | FcRn steady state affinity of VEGFang2-0016 (with AAA mutations): the concentration dependent binding response curve of VEGFang2-0016 (with AAA mutations) shows no binding to FcRn |
| **Figure 5E** | FcRn steady state affinity of VEGFang2-0016 (with AAA mutations): the concentration dependent binding response curve of VEGFang2-0016 (with AAA mutations) shows no binding to FcRn (Response range from -0.6 to 0.2 RU/ concentration scale ranges from 0 to 0.35 M) |
| **Figure 6** | FcgammaRIIIa interaction of VEGFang2-0015 without AAA mutations and VEGFang2-0016 with AAA mutations measurement (both are IgG1 subclass with P329G LALA mutations; as controls an Anti-Dig of IgG1 subclass and a IgG4 based antibody was used) |
| **Figure 7A** | Schematic Pk-ELISA Assay Principle for determination of concentrations of <VEGF/Ang2> bispecific antibodies in serum and whole eye lysates |
| **Figure 7B** | Serum concentration after intravenous application: Comparison of compounds -VEGFang2-0015 without AAA mutations and VEGFang2-0016 with AAA mutations |
| **Figure 7C** | Serum concentration after intravitreal application: Comparison of compounds -VEGFang2-0015 without AAA mutations and VEGFang2-0016 with AAA mutations |
| **Figure 7D** | Eye lysates concentration of VEGFang2-0016 (with AAA mutation) in right and left eye (after intravitreal application only into the right eye in comparison to intravenous application): Significant concentrations could be detected only in the right eye after intravitreal application. After intravenous application no concentrations in eye lysates could be detected due to the low serum half-life of VEGFang2-0016 (with AAA mutation) |
| **Figure 7E** | Eye lysates concentration of VEGFang2-0015 (without AAA mutation) in right and left eye (after intravitreal |

application only into the right eye in comparison to intravenous application): In the right eye ( and to some extent in the left eye) after intravitreal application concentrations of VEGFang2-0015 could be detected. This indicates the diffusion from the right eye into serum and from there into the left eye, which can be explained by the long half-life of VEGFang2-0015 (without AAA mutation). After intravenous application also significant concentrations in eye lysates of both eyes could be detected due to diffusion into the eyes of the serum-stable VEGFang2-0015 (without AAA mutation)

## Detailed Description of the Invention

In one embodiment of the invention the bispecific antibody according to the invention is bivalent.

In one aspect of the invention such bispecific, bivalent antibody according to the invention is characterized in comprising

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

This bispecific, bivalent antibody format for the bispecific antibody specifically binding to human vascular endothelial growth factor (VEGF) and human angiopoietin-2 (ANG-2) is described in WO 2009/080253 (including Knobs-into-Holes modified CH3 domains). The antibodies based on this bispecific, bivalent antibody format are named CrossMabs.

In one embodiment such bispecific, bivalent antibody is characterized in comprising

a) as heavy chain of the first full length antibody the amino acid sequence of SEQ ID NO: 25, and as light chain of the first full length antibody the amino acid sequence of SEQ ID NO: 27, and

b) as modified heavy chain of the second full length antibody the amino acid sequence of SEQ ID NO: 26, and as modified light chain of the second full length antibody the amino acid sequence of SEQ ID NO: 28.

In one embodiment such bispecific, bivalent antibody is characterized in comprising

a) as heavy chain of the first full length antibody the amino acid sequence of SEQ ID NO: 21, and as light chain of the first full length antibody the amino acid sequence of SEQ ID NO: 23, and
b) as modified heavy chain of the second full length antibody the amino acid sequence of SEQ ID NO: 22, and as modified light chain of the second full length antibody the amino acid sequence of SEQ ID NO: 24.

In one embodiment such bispecific, bivalent antibody is characterized in comprising

a) as heavy chain of the first full length antibody the amino acid sequence of SEQ ID NO: 29, and as light chain of the first full length antibody the amino acid sequence of SEQ ID NO: 31, and
b) as modified heavy chain of the second full length antibody the amino acid sequence of SEQ ID NO: 30, and as modified light chain of the second full length antibody the amino acid sequence of SEQ ID NO: 32.

Accordingly one embodiment of the invention 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: 25, of SEQ ID NO: 26, of SEQ ID NO: 27, and of SEQ ID NO: 28.

Accordingly one embodiment of the invention 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: 21, of SEQ ID NO: 22, of SEQ ID NO: 23, and of SEQ ID NO: 24.

Accordingly one embodiment of the invention 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.

In another aspect of the invention the bispecific antibody according to the invention is characterized in comprising

a) the heavy chain and the light chain of a first full length antibody that specifically binds to VEGF;
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 peptide linker.

This bispecific, bivalent antibody format for this bispecific antibody specifically binding to human vascular endothelial growth factor (VEGF) and human angiopoietin-2 (ANG-2) is described in WO 2011/117330 including Knobs-into-Holes modified CH3 domains. The antibodies based on this bispecific, bivalent antibody format are named OAscFabs.

In one embodiment such bispecific, bivalent antibody is characterized in comprising

a) as heavy chain of the first full length antibody the amino acid sequence of SEQ ID NO: 33, and as light chain of the first full length antibody the amino acid sequence of SEQ ID NO: 35, and
b) as heavy chain of the second full length antibody connected to the light chain of the second full length antibody via a peptide linker the amino acid sequence of SEQ ID NO: 34.

In one embodiment such bispecific, bivalent antibody is characterized in comprising

a) as heavy chain of the first full length antibody the amino acid sequence of SEQ ID NO: 36, and as light chain of the first full length antibody the amino acid sequence of SEQ ID NO: 38, and
b) as heavy chain of the second full length antibody connected to the light chain of the second full length antibody via a peptide linker the amino acid sequence of SEQ ID NO: 37.

In one embodiment 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 following positions: heavy chain variable domain position 44 to light chain variable domain position 100 (numbering always according to EU index of Kabat (Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)). Such further disulfide stabilization is achieved by the introduction of a disulfide bond between the variable domains VH and VL of the second full length antibody heavy and light chain. Techniques to introduce unnatural disulfide bridges for stabilization are described e.g. in WO 94/029350, Rajagopal, V., et al, Prot. Engin. 10 (1997) 1453-59; Kobayashi et al., Nuclear Medicine & Biology 25 (1998) 387-393; or Schmidt, M., et al., Oncogene 18 (1999) 1711-1721.

Accordingly one embodiment of the invention 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: 33, of SEQ ID NO: 34, and of SEQ ID NO: 35.

Accordingly one embodiment of the invention 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: 36, of SEQ ID NO: 37, and of SEQ ID NO: 38.

In on embodiment the CH3 domains of the bispecific, bivalent antibody according to the invention is altered by the “knob-into-holes” technology which is described in detail with several examples in e.g. WO 96/027011, Ridgway J.B., et al., Protein Eng 9 (1996) 617–621; and Merchant, A.M., et al., Nat Biotechnol 16 (1998) 677-681. In this method the interaction surfaces of the two CH3 domains are altered to increase the heterodimerisation of both heavy chains containing these two CH3 domains. Each of the two CH3 domains (of the two heavy chains) can be the “knob”, while the other is the “hole”. The introduction of a disulfide bridge stabilizes the heterodimers (Merchant, A.M, et al., Nature Biotech 16 (1998) 677-681; Atwell, S., et al. J. Mol. Biol. 270 (1997) 26–35) and increases the yield.

In a preferred aspect of the invention all bispecific antibodies according to the invention are characterized in that the CH3 domain of one heavy chain and the CH3 domain of the other heavy chain each meet at an interface which comprises an original interface between the antibody CH3 domains;

wherein said interface is altered to promote the formation of the bispecific antibody, wherein the alteration is characterized in that:

a) the CH3 domain of one heavy chain is altered,

so that within the original interface the CH3 domain of one heavy chain that meets the original interface of the CH3 domain of the other heavy chain within the bispecific antibody,

an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the interface of the CH3 domain of one heavy chain which is positionable in a cavity within the interface of the CH3 domain of the other heavy chain

and

b) the CH3 domain of the other heavy chain is altered,

so that within the original interface of the second CH3 domain that meets the original interface of the first CH3 domain within the bispecific antibody

an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the interface of the second CH3 domain within which a protuberance within the interface of the first CH3 domain is positionable.

Thus the antibody according to invention is preferably characterized in that

> the CH3 domain of the heavy chain of the full length antibody of a) and the CH3 domain of the heavy chain of the full length antibody of b) each meet at an interface which comprises an alteration in the original interface between the antibody CH3 domains;
>
> wherein i) in the CH3 domain of one heavy chain
>
> an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the interface of

the CH3 domain of one heavy chain which is positionable in a cavity within the interface of the CH3 domain of the other heavy chain

and wherein

ii) in the CH3 domain of the other heavy chain

an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the interface of the second CH3 domain within which a protuberance within the interface of the first CH3 domain is positionable.

Preferably said amino acid residue having a larger side chain volume is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y), tryptophan (W).

Preferably said amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine (S), threonine (T), valine (V).

In one aspect of the invention both CH3 domains are further altered by the introduction of cysteine (C) as amino acid in the corresponding positions of each CH3 domain such that a disulfide bridge between both CH3 domains can be formed.

In one embodiment, the bispecific antibody comprises a T366W mutation in the CH3 domain of the “knobs chain” and T366S, L368A, Y407V mutations in the CH3 domain of the “hole chain”. An additional interchain disulfide bridge between the CH3 domains can also be used (Merchant, A.M, et al., Nature Biotech 16 (1998) 677-681) e.g. by introducing a Y349C mutation into the CH3 domain of the “knobs chain” and a E356C mutation or a S354C mutation into the CH3 domain of the “hole chain”.

In another embodiment, the bispecific antibody according to the invention comprises Y349C, T366W mutations in one of the two CH3 domains and E356C, T366S, L368A, Y407V mutations in the other of the two CH3 domains. In a another preferred embodiment the bispecific antibody comprises Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains (the additional Y349C mutation in one CH3 domain and the additional E356C or S354C mutation in the other CH3 domain forming a interchain disulfide bridge) (numbering always according to EU index of Kabat (Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)). But also other knobs-in-holes technologies as described by EP 1 870 459 A1, can be used alternatively or additionally. Thus another example for the bispecific antibody are R409D; K370E mutations in the CH3 domain of the "knobs chain" and D399K; E357K mutations in the CH3 domain of the "hole chain" (numbering always according to EU index of Kabat (Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)).

In another embodiment the bispecific antibody comprises a T366W mutation in the CH3 domain of the "knobs chain" and T366S, L368A, Y407V mutations in the CH3 domain of the "hole chain" and additionally R409D; K370E mutations in the CH3 domain of the "knobs chain" and D399K; E357K mutations in the CH3 domain of the "hole chain".

In another embodiment the bispecific antibody comprises Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains or said trivalent, bispecific antibody comprises Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains and additionally R409D; K370E mutations in the CH3 domain of the "knobs chain" and D399K; E357K mutations in the CH3 domain of the "hole chain".

In one embodiment of the invention the bispecific antibody according to the invention is characterized in having one or more of the following properties (determined in assays as described in Example 6

- shows a lower serum concentration compared to corresponding bispecific antibody without the mutations described under iii) (96 hours after intravitreal application in mice, which are mouse FcRn deficient, but hemizygous transgenic for human FcRn);

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

In one embodiment the bispecific, bivalent antibody is characterized in 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 that

i) said first antigen-binding site comprises as heavy chain variable domain (VH) the SEQ ID NO: 7, and as light chain variable domain (VL) the SEQ ID NO: 8; and

ii) said second antigen-binding site comprises as heavy chain variable domain (VH) the SEQ ID NO: 15, and as light chain variable domain (VL) the SEQ ID NO: 16; and

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

and having one or more of the following properties (determined in assays as described in Example 6

- shows a lower serum concentration compared to corresponding bispecific antibody without the mutations described under iii) (96 hours after intravitreal application in mice, which are mouse FcRn deficient, but hemizygous transgenic for human FcRn);

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

In one embodiment the bispecific antibody is characterized in 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 that

i) said first antigen-binding site comprises as heavy chain variable domain (VH) the SEQ ID NO: 7 with 1, 2 or 3 amino acid residue substitutions, and as light chain variable domain (VL) the SEQ ID NO: 8 with 1, 2 or 3 amino acid residue substitutions; and

ii) said second antigen-binding site comprises as heavy chain variable domain (VH) the SEQ ID NO: 15 with 1, 2 or 3 amino acid residue substitutions, and as light chain variable domain (VL) the SEQ ID NO: with 1, 2 or 3 amino acid residue substitutions; and

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

and having one or more of the following properties (determined in assays as described in Example 6

- shows a lower serum concentration compared to corresponding bispecific antibody without the mutations described under iii) (96 hours after intravitreal application in mice, which are mouse FcRn deficient, but hemizygous transgenic for human FcRn);

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

As used herein, "antibody" refers to a binding protein that comprises antigen-binding sites. The terms “binding site” or “antigen-binding site” as used herein denotes the region(s) of an antibody molecule to which a ligand actually binds. The term “antigen-binding site” comprises an antibody heavy chain variable domains (VH) and an antibody light chain variable domains (VL) (pair of VH/VL).).

Antibody specificity refers to selective recognition of the antibody for a particular epitope of an antigen. Natural antibodies, for example, are monospecific.

“Bispecific antibodies” according to the invention are antibodies which have two different antigen-binding specificities. Antibodies of the present invention are specific for two different antigens, VEGF as first antigen and ANG-2 as second antigen.

The term “monospecific” antibody as used herein denotes an antibody that has one or more binding sites each of which bind to the same epitope of the same antigen.

The term “valent” as used within the current application denotes the presence of a specified number of binding sites in an antibody molecule. As such, the terms “bivalent”, “tetravalent”, and “hexavalent” denote the presence of two binding site, four binding sites, and six binding sites, respectively, in an antibody molecule. The bispecific antibodies according to the invention are preferably “bivalent”.

The term “VEGF” as used herein refers to human vascular endothelial growth factor (VEGF/VEGF-A,) the 165-amino acid human vascular endothelial cell growth factor (amino acid 27-191 of precursor sequence of human VEGF165: SEQ ID NO: 17; amino acids 1-26 represent the signal peptide), and related 121, 189, and 206 vascular endothelial cell growth factor isoforms, as described by Leung, D.W., et al., Science 246 (1989) 1306-9; Houck et al., Mol. Endocrin. 5 ( 1991) 1806 -1814; Keck, P.J., et al., Science 246 (1989) 1309-12 and Connolly, D.T., et al., J. Biol. Chem. 264 (1989) 20017-24; together with the naturally occurring allelic and processed forms of those growth factors. VEGF is involved in the regulation of normal and abnormal angiogenesis and neovascularization associated with tumors and intraocular disorders (Ferrara, N., et al., Endocr. Rev. 18 (1997) 4-25; Berkman, R.A.,et al., J. Clin. Invest. 91 (1993) 153-159; Brown, L.F., et al., Human Pathol. 26 (1995) 86-91; Brown, L.F., et al., Cancer Res. 53 (1993) 4727-4735; Mattern, J., et al., Brit. J. Cancer. 73 (1996) 931-934; and Dvorak, H.F., et al., Am. J. Pathol. 146 (1995) 1029-1039). VEGF is a homodimeric glycoprotein that has been isolated from several sources and includes several isoforms. VEGF shows highly specific mitogenic activity for endothelial cells.

The term “ANG-2” as used herein refers to human angiopoietin-2 (ANG-2) (alternatively abbreviated with ANGPT2 or ANG2) (SEQ ID NO: 18) which is described e.g. in Maisonpierre, P.C., et al, Science 277 (1997) 55-60 and Cheung, A.H., et al., Genomics 48 (1998) 389-91. The angiopoietins-1 (SEQ ID NO: 19) and -2 were discovered as ligands for the Ties, a family of tyrosine kinases that is selectively expressed within the vascular endothelium (Yancopoulos, G.D., et al., Nature 407 (2000) 242-48). There are now four definitive members of the angiopoietin family. Angiopoietin-3 and -4 (Ang-3 and Ang-4) may represent widely diverged counterparts of the same gene locus in mouse and man (Kim, I., et al., FEBS Let, 443 (1999) 353-56; Kim, I., et al., J Biol Chem 274 (1999) 26523-28). ANG-1 and ANG-2 were originally identified in tissue culture experiments as agonist and antagonist, respectively (see for ANG-1: Davis, S., et al., Cell 87 (1996) 1161-69; and for ANG-2: Maisonpierre, P.C., et al., Science 277 (1997) 55-60). All of the known angiopoietins bind primarily to Tie2 (SEQ ID NO: 20), and both Ang-1 and -2 bind to Tie2 with an affinity of 3 nM (Kd) (Maisonpierre, P.C., et al., Science 277 (1997) 55-60).

An antigen-binding sites of the bispecific antibody of the invention contain six complementarity determining regions (CDRs) which contribute in varying degrees to the affinity of the binding site for antigen. There are three heavy chain variable domain CDRs (CDRH1, CDRH2 and CDRH3) and three light chain variable domain CDRs (CDRL1, CDRL2 and CDRL3). The extent of CDR and framework regions (FRs) is determined by comparison to a compiled database of amino acid sequences in which those regions have been defined according to variability among the sequences.

The antibodies of the invention comprise immunoglobulin constant regions derived from human origin of one or more immunoglobulin classes, wherein such . immunoglobulin classes include IgG, IgM, IgA, IgD, and IgE classes and, in the case of IgG and IgA, their subclasses, especially IgG1 and IgG4..

The terms "monoclonal antibody" or "monoclonal antibody composition” as used herein refer to a preparation of antibody molecules of a single amino acid composition.

The term "chimeric antibody" refers to an antibody comprising a variable region, i.e., binding region, from one source or species and at least a portion of a constant region derived from a different source or species, usually prepared by recombinant DNA techniques. Chimeric antibodies comprising a murine variable region and a human constant region are preferred. Other preferred forms of "chimeric antibodies" encompassed by the present invention are those in which the constant region has been modified or changed from that of the original antibody to generate the properties according to the invention, especially in regard to C1q binding and/or Fc receptor (FcR) binding. Such chimeric antibodies are also referred to as "class-switched antibodies.". Chimeric antibodies are the product of expressed immunoglobulin genes comprising DNA segments encoding immunoglobulin variable regions and DNA segments encoding immunoglobulin constant regions. Methods for producing chimeric antibodies involve conventional recombinant DNA and gene transfection techniques are well known in the art. See, e.g., Morrison, S.L., et al., Proc. Natl. Acad. Sci. USA 81 (1984) 6851-6855; US 5,202,238 and US 5,204,244.

The term "humanized antibody" refers to antibodies in which the framework or "complementarity determining regions" (CDR) have been modified to comprise the CDR of an immunoglobulin of different specificity as compared to that of the parent immunoglobulin. In a preferred embodiment, a murine CDR is grafted into the framework region of a human antibody to prepare the "humanized antibody." See, e.g., Riechmann, L., et al., Nature 332 (1988) 323-327; and Neuberger, M.S., et al., Nature 314 (1985) 268-270. Particularly preferred CDRs correspond to those representing sequences recognizing the antigens noted above for chimeric antibodies. Other forms of "humanized antibodies" encompassed by the present invention are those in which the constant region has been additionally modified or changed from that of the original antibody to generate the properties according to the invention, especially in regard to C1q binding and/or Fc receptor (FcR) binding.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germ line immunoglobulin sequences. Human antibodies are well-known in the state of the art (van Dijk, M.A., and van de Winkel, J.G., Curr. Opin. Chem. Biol. 5 (2001) 368-374). Human antibodies can also be produced in transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire or a selection of human antibodies in the absence of endogenous immunoglobulin production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (see, e.g., Jakobovits, A., et al., Proc. Natl. Acad. Sci. USA 90 (1993) 2551-2555; Jakobovits, A., et al., Nature 362 (1993) 255-258; Brueggemann, M., et al., Year Immunol. 7 (1993) 33-40). Human antibodies can also be produced in phage display libraries (Hoogenboom, H.R., and Winter, G., J. Mol. Biol. 227 (1992) 381-388; Marks, J.D., et al., J. Mol. Biol. 222 (1991) 581-597). The techniques of Cole, A., et al. and Boerner, P., et al. are also available for the preparation of human monoclonal antibodies (Cole, A., et al., Monoclonal Antibodies and Cancer Therapy, Liss, A.L., p. 77 (1985); and Boerner, P., et al., J. Immunol. 147 (1991) 86-95). As already mentioned for chimeric and humanized antibodies according to the invention the term “human antibody” as used herein also comprises such antibodies which are modified in the constant region to generate the properties according to the invention, especially in regard to C1q binding and/or FcR binding, e.g. by “class switching” i.e. change or mutation of Fc parts (e.g. from IgG1 to IgG4 and/or IgG1/IgG4 mutation).

The term "recombinant antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from a host cell such as a NS0 or CHO cell or from an animal (e.g. a mouse) that is transgenic for human immunoglobulin genes or antibodies expressed using a recombinant expression vector transfected into a host cell. Such recombinant antibodies have variable and constant regions in a rearranged form. The recombinant antibodies according to the invention have been subjected to in vivo somatic hypermutation. Thus, the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germ line VH and VL sequences, may not naturally exist within the human antibody germ line repertoire in vivo.

The "variable domain" (variable domain of a light chain (VL), variable domain of a heavy chain (VH) as used herein denotes each of the pair of light and heavy chains which is involved directly in binding the antibody to the antigen. The domains of variable human light and heavy chains have the same general structure and each domain comprises four framework (FR) regions whose sequences are widely conserved, connected by three "hypervariable regions" (or complementarity determining regions, CDRs). The framework regions adopt a β-sheet conformation and the CDRs may form loops connecting the β-sheet structure. The CDRs in each chain are held in their three-dimensional structure by the framework regions and form together with the CDRs from the other chain the antigen binding site. The antibody heavy and light chain CDR3 regions play a particularly important role in the binding specificity/affinity of the antibodies according to the invention and therefore provide a further object of the invention.

The terms "hypervariable region" or "antigen-binding portion of an antibody" when used herein refer to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from the "complementarity determining regions" or "CDRs". "Framework" or "FR" regions are those variable domain regions other than the hypervariable region residues as herein defined. Therefore, the light and heavy chains of an antibody comprise from N- to C-terminus the domains FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. CDRs on each chain are separated by such framework amino acids. Especially, CDR3 of the heavy chain is the region which contributes most to antigen binding. CDR and FR regions are determined according to the standard definition of Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991).

As used herein, the term “binding” or “specifically binding” refers to the binding of the antibody to an epitope of the antigen (either human VEGF or human ANG-2) in an in vitro assay, preferably in an plasmon resonance assay (BIAcore, GE-Healthcare Uppsala, Sweden with purified wild-type antigen. The affinity of the binding is defined by the terms ka (rate constant for the association of the antibody from the antibody/antigen complex), $k_D$ (dissociation constant), and $K_D$ ($k_D$/ka). In one embodiment binding or specifically binding means a binding affinity ($K_D$) of $10^{-8}$ mol/l or less, in one embodiment $10^{-9}$ M to $10^{-13}$ mol/l.

The term "epitope" includes any polypeptide determinant capable of specific binding to an antibody. In certain embodiments, epitope determinant include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody.

In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules.

The term “full length antibody” denotes an antibody consisting of two “full length antibody heavy chains” and two “full length antibody light chains”. A “full length antibody heavy chain” is a polypeptide consisting in N-terminal to C-terminal direction of an antibody heavy chain variable domain (VH), an antibody constant heavy chain domain 1 (CH1), an antibody hinge region (HR), an antibody heavy chain constant domain 2 (CH2), and an antibody heavy chain constant domain 3 (CH3), abbreviated as VH-CH1-HR-CH2-CH3; and optionally an antibody heavy chain constant domain 4 (CH4) in case of an antibody of the subclass IgE. Preferably the “full length antibody heavy chain” is a polypeptide consisting in N-terminal to C-terminal direction of VH, CH1, HR, CH2 and CH3. A “full length antibody light chain” is a polypeptide consisting in N-terminal to C-terminal direction of an antibody light chain variable domain (VL), and an antibody light chain constant domain (CL), abbreviated as VL-CL. The antibody light chain constant domain (CL) can be κ (kappa) or λ (lambda). The two full length antibody chains are linked together via inter-polypeptide disulfide bonds between the CL domain and the CH1 domain and between the hinge regions of the full length antibody heavy chains. Examples of typical full length antibodies are natural antibodies like IgG (e.g. IgG 1 and IgG2), IgM, IgA, IgD, and IgE. The full length antibodies according to the invention can be from a single species e.g. human, or they can be chimerized or humanized antibodies. The full length antibodies according to the invention comprise two antigen binding sites each formed by a pair of VH and VL, which both specifically bind to the same antigen. The C-terminus of the heavy or light chain of said full length antibody denotes the last amino acid at the C-terminus of said heavy or light chain. The N-terminus of the heavy or light chain of said full length antibody denotes the last amino acid at the N- terminus of said heavy or light chain.

The term “peptide linker” as used within the invention denotes a peptide with amino acid sequences, which is preferably of synthetic origin. These peptides according to invention are used to connect the C-terminus of the light chain to the N-terminus of heavy chain of the second full length antibody (that specifically binds to a second antigen) via a peptide linker. The peptide linker within the second full length antibody heavy and light chain is a peptide with an amino acid sequence with a length of at least 30 amino acids, preferably with a length of 32 to 50 amino acids. In one the peptide linker is a peptide with an amino acid sequence with a length of 32 to 40 amino acids. In one embodiment said linker is (GxS)n with G = glycine, S = serine, (x =3, n= 8, 9 or 10 and m= 0, 1, 2 or 3) or (x = 4 and n= 6, 7 or 8 and m= 0, 1, 2 or 3), preferably with x = 4, n= 6 or 7 and m= 0, 1, 2 or 3, more preferably with x = 4, n= 7 and m= 2. In one embodiment said linker is $(G_4S)_6G_2$.

The term “constant region” as used within the current applications denotes the sum of the domains of an antibody other than the variable region. The constant region is not involved directly in binding of an antigen, but exhibits various effector functions. Depending on the amino acid sequence of the constant region of their heavy chains, antibodies are divided in the classes: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses, such as IgG1, IgG2, IgG3, and IgG4, IgA1 and IgA2. The heavy chain constant regions that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively. The light chain constant regions which can be found in all five antibody classes are called κ (kappa) and λ (lambda).

The terms "constant region derived from human origin" or " human constant region" as used in the current application denotes a constant heavy chain region of a human antibody of the subclass IgG1, IgG2, IgG3, or IgG4 and/or a constant light chain kappa or lambda region. Such constant regions are well known in the state of the art and e.g. described by Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) (see also e.g. Johnson, G., and Wu, T.T., Nucleic Acids Res. 28 (2000) 214-218; Kabat, E.A., et al., Proc. Natl. Acad. Sci. USA 72 (1975) 2785-2788). Within the application for the numbering of positions and mutations the EU numbering system (EU Index) according to Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) is used and referred to as "numbering according to EU Index of Kabat".

In one embodiment the bispecific antibodies according to the invention have a constant region of human IgG1 subclass (derived from human IgG1 subclass) .

In one embodiment the bispecific antibodies according to the invention have a constant region of human IgG4 subclass (derived from human IgG1 subclass).

In one embodiment the bispecific antibody according to the invention is of human IgG1 subclass with mutations L234A (Leu235Ala), L235A (Leu234Ala) and P329G (Pro329Gly). Such antibody has a reduced FcR binding (especially they show no more binding to FcRgammaI, FcRgammaII and FcRgammaIII). This especially useful to reduce potential side effects like e.g. thrombosis (Meyer, T., et al., J. Thromb. Haemost. 7 (2009) 171-81). In one embodiment the bispecific antibody according to the invention is of human IgG4 subclass with mutations S228P (Ser228Pro), L235E (Leu235Glu) and P329G (Pro329Gly). Such antibody shows reduced FcR binding as indicated above. While Pro329Ala mutation which was described already removes only two third of the FcgammaRIIIa sandwich interaction, the Pro329Gly in the antibodies according to the invention fully imparts binding of the Fc part to FcgammaRIII. This is especially useful as the binding to FcgammaRIII is involved in ADCC (antibody –dependent cellular toxicity) which leads to cell death, which may be helpful in the treatment of cancer diseases, but which can cause serious side effect in the antibody based treatment of other vascular or immunological diseases. So the antibodies according to the invention of IgG1 subclass with mutations L234A, L235A and P329G and IgG4 subclass with mutations S228P, L235E and P329G are especially useful, as they both show no more binding to FcRgammaI, FcRgammaII and FcRgammaIII.

The term "with (the) mutations AAA" as used herein refers the mutations I253A (Ile253Ala), H310A (His310Ala), and H435A (His435Ala) in the constant heavy chain region of IgG1 or IgG4, wherein the numbering is according to the EU Index of Kabat.

The term "with (the) mutations P329G LALA" as used herein refers to the mutations L234A (Leu235Ala) , L235A (Leu234Ala) and P329G (Pro329Gly) in the constant heavy chain region of IgG1 subclass, wherein the numbering is according to the EU Index of Kabat. The term "with (the) mutations SPLE" as used herein refers to the S228P (Ser228Pro) and L235E (Leu235Glu) the constant heavy chain region of IgG4 subclass, wherein the numbering is according to the EU Index of Kabat. The term "with (the) mutations SPLE and P239G" as used herein refers to the S228P (Ser228Pro), L235E (Leu235Glu) and P329G (Pro329Gly) the constant heavy chain region of IgG4 subclass, wherein the numbering is according to the EU Index of Kabat.

The antibody according to the invention is produced by recombinant means. Thus, one aspect of the current invention is a nucleic acid encoding the antibody according to the invention and a further aspect is a cell comprising said nucleic acid encoding an antibody according to the invention. Methods for recombinant production are widely known in the state of the art and comprise protein expression in prokaryotic and eukaryotic cells with subsequent isolation of the antibody and usually purification to a pharmaceutically acceptable purity. For the expression of the antibodies as aforementioned in a host cell, nucleic acids encoding the respective modified light and heavy chains are inserted into expression vectors by standard methods. Expression is performed in appropriate prokaryotic or eukaryotic host cells like CHO cells, NS0 cells, SP2/0 cells, HEK293 cells, COS cells, PER.C6 cells, yeast, or E.coli cells, and the antibody is recovered from the cells (supernatant or cells after lysis). General methods for recombinant production of antibodies are well-known in the state of the art and described, for example, in the review articles of Makrides, S.C., Protein Expr. Purif. 17 (1999) 183-202; Geisse, S., et al., Protein Expr. Purif. 8 (1996) 271-282; Kaufman, R.J., Mol. Biotechnol. 16 (2000) 151-160; Werner, R.G., Drug Res. 48 (1998) 870-880.

Accordingly one embodiment of the invention is a method for the preparation of a bispecific antibody according to the invention, 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.

In one embodiment the recovering step under c includes the use of a light chain constant domain specific capture reagent ( which e.g. specific for the kappa or the lambda constant light chain, depending on whether a kappa or a lambda light chain in the bispecific antibody according to invention used). In one embodiment this light chain specific capture reagent is used in in a bind-and-elute-mode). Examples of such light chain constant domain specific capture reagents are e.g. KappaSelect™ and LambdaFabSelectTM from GE Healthcare/BAC, which are based on a highly rigid agarose base matrix that allows high flow rates and low back pressure at large scale. They feature a ligand that binds to the constant region of the kappa or the lambda light chain respectively (i.e. fragments lacking the constant region of the light chain will not bind; Fig 1). Both are therefore capable of binding other target molecules containing the constant region of the light chain, for example, IgG, IgA and IgM. The ligands are attached to the matrix via a long hydrophilic spacer arm to make it easily available for binding to the target molecule. They are based on a single-chain antibody fragment that is screened for either human Ig kappa or lambda.

The bispecific antibodies are suitably separated from the culture medium by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography. DNA and RNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures. The hybridoma cells can serve as a source of such DNA and RNA. Once isolated, the DNA may be inserted into expression vectors, which are then transfected into host cells such as HEK 293 cells, CHO cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of recombinant monoclonal antibodies in the host cells.

Amino acid sequence variants (or mutants) of the bispecific antibody are prepared by introducing appropriate nucleotide changes into the antibody DNA, or by nucleotide synthesis. Such modifications can be performed, however, only in a very limited range. For example, the modifications do not alter the above mentioned antibody characteristics such as the IgG subclass and antigen binding, but may improve the yield of the recombinant production, protein stability or facilitate the purification.

The term “host cell” as used in the current application denotes any kind of cellular system which can be engineered to generate the antibodies according to the current invention. In one embodiment HEK293 cells and CHO cells are used as host cells. As used herein, the expressions “cell,” “cell line,” and “cell culture” are used interchangeably and all such designations include progeny. Thus, the words “transformants” and “transformed cells” include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological activity as screened for in the originally transformed cell are included.

Expression in NS0 cells is described by, e.g., Barnes, L.M., et al., Cytotechnology 32 (2000) 109-123; Barnes, L.M., et al., Biotech. Bioeng. 73 (2001) 261-270. Transient expression is described by, e.g., Durocher, Y., et al., Nucl. Acids. Res. 30 (2002) E9. Cloning of variable domains is described by Orlandi, R., et al., Proc. Natl. Acad. Sci. USA 86 (1989) 3833-3837; Carter, P., et al., Proc. Natl. Acad. Sci. USA 89 (1992) 4285-4289; and Norderhaug, L., et al., J. Immunol. Methods 204 (1997) 77-87. A preferred transient expression system (HEK 293) is described by Schlaeger, E.-J., and Christensen, K., in Cytotechnology 30 (1999) 71-83 and by Schlaeger, E.-J., in J. Immunol. Methods 194 (1996) 191-199.

The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, enhancers and polyadenylation signals.

A nucleic acid is “operably linked” when it is placed in a functional relationship with another nucleic acid sequence. For example, DNA for a pre-sequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a pre-protein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, “operably linked” means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

Purification of antibodies is performed in order to eliminate cellular components or other contaminants, e.g. other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis, and others well known in the art. See Ausubel, F., et al., ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987). Different methods are well established and widespread used for protein purification, such as affinity chromatography with microbial proteins (e.g. protein A or protein G affinity chromatography), ion exchange chromatography (e.g. cation exchange (carboxymethyl resins), anion exchange (amino ethyl resins) and mixed-mode exchange), thiophilic adsorption (e.g. with beta-mercaptoethanol and other SH ligands), hydrophobic interaction or aromatic adsorption chromatography (e.g. with phenyl-sepharose, aza-arenophilic resins, or m-aminophenylboronic acid), metal chelate affinity chromatography (e.g. with Ni(II)- and Cu(II)-affinity material), size exclusion chromatography, and electrophoretical methods (such as gel electrophoresis, capillary electrophoresis) (Vijayalakshmi, M.A., Appl. Biochem. Biotech. 75 (1998) 93-102).

The bispecific, bivalent antibodies according to the invention show benefits for human patients in need of a VEGF and ANG-2 targeting therapy.

The bivalent bispecific against human VEGF and human ANG-2 according to the current invention may have a valuable efficacy/safety profile and may provide benefits for a patient in the need of an anti-VEGF and anti-ANG-2 therapy.

One aspect of the invention is a pharmaceutical composition comprising an antibody according to the invention. Another aspect of the invention is the use of an antibody according to the invention for the manufacture of a pharmaceutical composition. A further aspect of the invention is a method for the manufacture of a pharmaceutical composition comprising an antibody according to the invention. In another aspect, the present invention provides a composition, e.g. a pharmaceutical composition, containing an antibody according to the present invention, formulated together with a pharmaceutical carrier.

As used herein, “pharmaceutical carrier” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for administration administered to the subject via a local route.. For example, the antibody or its composition can be administered to the subject by intraocular application e.g. by intraocular injection such as intravitreal injection. This can be performed in accordance with standard procedures known in the art. See, e.g., Ritter et al., J. Clin. Invest. 116 (2006) 3266-76; Russelakis-Carneiro et al., Neuropathol. Appl. Neurobiol. 25 (1999) 196-206; and Wray et al., Arch. Neurol. 33 (1976) 183-5.

A composition of the present invention can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. To administer a compound of the invention by certain routes of administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Pharmaceutical carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art.

Many possible modes of delivery can be used, including, but not limited to intraocular application or topical application. In one embodiment the application is intraocular and includes, but is not limited to, subconjunctival injection, intracanieral injection, injection into the anterior chamber via the termporal limbus, intrastromal injection, intracorneal injection, subretinal injection, aqueous humor injection, subtenon injection or sustained delivery device, intravitreal injection (e.g., front, mid or back vitreal injection). In one embodiment the application is topical and includes, but is not limited to eye drops to the cornea.

In one embodiment the bispecific antibody or pharmaceutical composition according to the invention is administered via intravitreal application, e.g. via intravitreal injection. This can be performed in accordance with standard procedures known in the art. See, e.g., Ritter et al., J. Clin. Invest. 116 (2006) 3266-76; Russelakis-Carneiro et al., Neuropathol. Appl. Neurobiol. 25 (1999) 196-206; and Wray et al., Arch. Neurol. 33 (1976) 183-5.

In some embodiments, therapeutic kits of the invention can contain one or more doses of a bispecific antibody present in a pharmaceutical composition described herein, a suitable device for intravitreal injection of the pharmaceutical composition, and an instruction detailing suitable subjects and protocols for carrying out the injection. In these embodiments, the compositions are typically administered to the subject in need of treatment via intravitreal injection. This can be performed in accordance with standard procedures known in the art. See, e.g., Ritter et al., J. Clin. Invest. 116 (2006) 3266-76; Russelakis-Carneiro et al., Neuropathol. Appl. Neurobiol. 25 (1999) 196-206; and Wray et al., Arch. Neurol. 33 (1976) 183-5.

The compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

The composition must be sterile and fluid to the extent that the composition is deliverable by syringe. In addition to water, the carrier preferably is an isotonic buffered saline solution.

Proper fluidity can be maintained, for example, by use of coating such as lecithin, by maintenance of required particle size in the case of dispersion and by use of surfactants. In many cases, it is preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol or sorbitol, and sodium chloride in the composition.

The composition can comprise an ophthalmic depot formulation comprising an active agent for subconjunctival administration. The ophthalmic depot formulation comprises microparticles of essentially pure active agent, e.g., the bispecific antibody according to the invention. The microparticles comprising the bispecific antibody according to the invention can be embedded in a biocompatible pharmaceutically acceptable polymer or a lipid encapsulating agent. The depot formulations may be adapted to release all of substantially all the active material over an extended period of time. The polymer or lipid matrix, if present, may be adapted to degrade sufficiently to be transported from the site of administration after release of all or substantially all the active agent. The depot formulation can be liquid formulation, comprising a pharmaceutical acceptable polymer and a dissolved or dispersed active agent. Upon injection, the polymer forms a depot at the injections site, e.g. by gelifying or precipitating.

Another aspect of the invention is the bispecific antibody according to the invention for use in the treatment of ocular vascular diseases.

One embodiment of the invention is the bispecific antibody according to the invention for use in the treatment of ocular vascular diseases.

Another aspect of the invention is said pharmaceutical composition for use in the treatment of ocular vascular diseases.

Another aspect of the invention is the use of an antibody according to the invention for the manufacture of a medicament for the treatment of ocular vascular disease.

Another aspect of the invention is method of treatment of patient suffering from ocular vascular diseases by administering an antibody according to the invention to a patient in the need of such treatment.

The terms “ocular vascular disease” and “vascular eye disease” are use inter changeable herein and include, but are not limited to intraocular neovascular syndromes such as diabetic retinopathy, diabetic macular edema,, retinopathy of prematurity, neovascular glaucoma, retinal vein occlusions, central retinal vein occlusions, macular degeneration, age-related macular degeneration, retinitis pigmentosa, retinal angiomatous proliferation, macular telangectasia, ischemic retinopathy, iris neovascularization, intraocular neovascularization, corneal neovascularization, retinal neovascularization, choroidal neovascularization, and retinal degeneration. (Garner, A., Vascular diseases, In: Pathobiology of ocular disease, A dynamic approach, Garner, A., and Klintworth, G.K., (eds.), 2nd edition, Marcel Dekker, New York (1994), pp. 1625-1710). As used herein, ocular vascular disorder refers to any pathological conditions characterized by altered or unregulated proliferation and invasion of new blood vessels into the structures of ocular tissues such as the retina or cornea. In one embodiment the ocular vascular disease is selected from the group consisting of: wet age-related macular degeneration (wet AMD), dry age-related macular degeneration (dry AMD), diabetic macular edema (DME), cystoid macular edema (CME), non-proliferative diabetic retinopathy (NPDR), proliferative diabetic retinopathy (PDR), cystoid macular edema, vasculitis (e.g. central retinal vein occlusion), papilloedema, retinitis, conjunctivitis, uveitis, choroiditis, multifocal choroiditis, ocular histoplasmosis, blepharitis, dry eye (Sjögren's disease) and other ophthalmic diseases wherein the eye disease or disorder is associated with ocular neovascularization, vascular leakage, and/or retinal edema. So the bispecific antibodies according to the invention are useful in the prevention and treatment of wet AMD, dry AMD, CME, DME, NPDR, PDR, blepharitis, dry eye and uveitis, also preferably wet AMD, dry AMD, blepharitis, and dry eye, also preferably CME, DME, NPDR and PDR, also preferably blepharitis, and dry eye, in particular wet AMD and dry AMD, and also particularly wet AMD. In some embodiments, the ocular disease is selected from the group consisting of wet age-related macular degeneration (wet AMD), macular edema, retinal vein occlusions, retinopathy of prematurity, and diabetic retinopathy.

Other diseases associated with corneal neovascularization include, but are not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogrens, acne rosacea, phylectenulosis, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, Herpes simplex infections, Herpes zoster infections, protozoan infections, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, mariginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, periphigoid radial keratotomy, and corneal graph rejection.

Diseases associated with retinal/choroidal neovascularization include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosis, retinopathy of prematurity, retinitis pigmentosa, retina edema (including macular edema), Eales disease, Bechets disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Bests disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy.

Retinopathy of prematurity (ROP) is a disease of the eye that affects prematurely born babies. It is thought to be caused by disorganized growth of retinal blood vessels which may result in scarring and retinal detachment. ROP can be mild and may resolve spontaneously, but may lead to blindness in serious cases. As such, all preterm babies are at risk for ROP, and very low birth weight is an additional risk factor. Both oxygen toxicity and relative hypoxia can contribute to the development of ROP.

Macular degeneration is a medical condition predominantly found in elderly adults in which the center of the inner lining of the eye, known as the macula area of the retina, suffers thinning, atrophy, and in some cases, bleeding. This can result in loss of central vision, which entails inability to see fine details, to read, or to recognize faces. According to the American Academy of Ophthalmology, it is the leading cause of central vision loss (blindness) in the United States today for those over the age of fifty years. Although some macular dystrophies that affect younger individuals are sometimes referred to as macular degeneration, the term generally refers to age-related macular degeneration (AMD or ARMD).

Age-related macular degeneration begins with characteristic yellow deposits in the macula (central area of the retina which provides detailed central vision, called fovea) called drusen between the retinal pigment epithelium and the underlying choroid. Most people with these early changes (referred to as age-related maculopathy) have good vision. People with drusen can go on to develop advanced AMD. The risk is considerably higher when the drusen are large and numerous and associated with disturbance in the pigmented cell layer under the macula. Large and soft drusen are related to elevated cholesterol deposits and may respond to cholesterol lowering agents or the Rheo Procedure.

Advanced AMD, which is responsible for profound vision loss, has two forms: dry and wet. Central geographic atrophy, the dry form of advanced AMD, results from atrophy to the retinal pigment epithelial layer below the retina, which causes vision loss through loss of photoreceptors (rods and cones) in the central part of the eye. While no treatment is available for this condition, vitamin supplements with high doses of antioxidants, lutein and zeaxanthin, have been demonstrated by the National Eye Institute and others to slow the progression of dry macular degeneration and in some patients, improve visual acuity.

Retinitis pigmentosa (RP) is a group of genetic eye conditions. In the progression of symptoms for RP, night blindness generally precedes tunnel vision by years or even decades. Many people with RP do not become legally blind until their 40s or 50s and retain some sight all their life. Others go completely blind from RP, in some cases as early as childhood. Progression of RP is different in each case. RP is a type of hereditary retinal dystrophy, a group of inherited disorders in which abnormalities of the photoreceptors (rods and cones) or the retinal pigment epithelium (RPE) of the retina lead to progressive visual loss. Affected individuals first experience defective dark adaptation or nyctalopia (night blindness), followed by reduction of the peripheral visual field (known as tunnel vision) and, sometimes, loss of central vision late in the course of the disease.

Macular edema occurs when fluid and protein deposits collect on or under the macula of the eye, a yellow central area of the retina, causing it to thicken and swell. The swelling may distort a person's central vision, as the macula is near the center of the retina at the back of the eyeball. This area holds tightly packed cones that provide sharp, clear central vision to enable a person to see form, color, and detail that is directly in the line of sight. Cystoid macular edema is a type of macular edema that includes cyst formation.

Combination Therapies: In certain embodiments the bispecific antibody or pharmaceutical composition according to the invention is administered alone (without an additional therapeutic agent) for the treatment of one or more ocular vascular diseases described herein.

In other embodiments the bispecific antibody or pharmaceutical composition according to the invention is administered in combination with one or more additional therapeutic agents or methods for the treatment of one or more ocular vascular diseases described herein.

In other embodiments, the bispecific antibody or pharmaceutical composition according to the invention is formulated in combination with one or more additional therapeutic agents and administered for the treatment of one or more ocular vascular diseases described herein.

In certain embodiments, the combination treatments provided herein include administration the bispecific antibody or pharmaceutical composition according to the invention is administered sequentially with one or more additional therapeutic agents for the treatment of one or more ocular vascular diseases described herein.

The additional therapeutic agents include, but are not limited to, Tryptophanyl-tRNA synthetase (TrpRS), EyeOOl (Anti-VEGF Pegylated Aptamer), squalamine, RETAANE(TM) (anecortave acetate for depot suspension; Alcon, Inc.), Combretastatin A4 Prodrug (CA4P), MACUGEN(TM), MIFEPREX(TM) (mifepristone-ru486), subtenon triamcinolone acetonide, intravitreal crystalline triamcinolone acetonide, Prinomastat (AG3340- synthetic matrix metalloproteinase inhibitor, Pfizer), fluocinolone acetonide (including fluocinolone intraocular implant, Bausch & Lomb/Control Delivery Systems), VEGFR inhibitors (Sugen), VEGF-Trap (Regeneron/Aventis), VEGF receptor tyrosine kinase inhibitors such as 4-(4-bromo- 2-fluoroanilino)-6- methoxy-7-(l-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474), 4-(4-fluoro-2-methylindol-5- yloxy)-6-methoxy-7-(3- pyrrolidin- 1 -ylpropoxy)quinazoline (AZD2171), vatalanib (PTK787) and SU1 1248 (sunitinib), linomide, and inhibitors of integrin v.beta.3 function and angiostatin.

Other pharmaceutical therapies that can be sued used in combination the bispecific antibody or pharmaceutical composition according to the invention is administered, include, but are not limited to, VISUDYNE(TM) with use of a non-thermal laser, PKC 412, Endovion (NeuroSearch A/S), neurotrophic factors, including by way of example Glial Derived Neurotrophic Factor and Ciliary Neurotrophic Factor, diatazem, dorzolamide, Phototrop, 9-cis-retinal, eye medication (including Echo Therapy) including phospholine iodide or echothiophate or carbonic anhydrase inhibitors, AE-941 (AEterna Laboratories, Inc.), Sirna-027 (Sima Therapeutics, Inc.), pegaptanib (NeXstar Pharmaceuticals/Gilead Sciences), neurotrophins (including, by way of example only, NT-4/5, Genentech), Cand5 (Acuity Pharmaceuticals), INS-37217 (Inspire Pharmaceuticals), integrin antagonists (including those from Jerini AG and Abbott Laboratories), EG-3306 (Ark Therapeutics Ltd.), BDM-E (BioDiem Ltd.), thalidomide (as used, for example, by EntreMed, Inc.), cardiotrophin-1 (Genentech), 2-methoxyestradiol (Allergan/Oculex), DL-8234 (Toray Industries), NTC-200 (Neurotech), tetrathiomolybdate (University of Michigan), LYN-002 (Lynkeus Biotech), microalgal compound (Aquasearch/ Albany, Mera Pharmaceuticals), D-9120 (Celltech Group pic), ATX-S10 (Hamamatsu Photonics), TGF-beta 2 (Genzyme/Celtrix), tyrosine kinase inhibitors (Allergan, SUGEN, Pfizer), NX-278-L (NeXstar Pharmaceuticals/Gilead Sciences), Opt-24 (OPTIS France SA), retinal cell ganglion neuroprotectants (Cogent Neurosciences), N- nitropyrazole derivatives (Texas A&M University System), KP-102 (Krenitsky Pharmaceuticals), cyclosporin A, Timited retinal translocation", photodynamic therapy, (including, by way of example only, receptor-targeted PDT, Bristol-Myers Squibb, Co.; porfimer sodium for injection with PDT; verteporfin, QLT Inc.; rostaporfin with PDT, Miravent Medical Technologies; talaporfin sodium with PDT, Nippon Petroleum; motexafin lutetium, Pharmacyclics, Inc.), antisense oligonucleotides (including, by way of example, products tested by Novagali Pharma SA and ISIS-13650, Isis Pharmaceuticals), laser photocoagulation, drusen lasering, macular hole surgery, macular translocation surgery, implantable miniature telescopes, Phi-Motion Angiography (also known as Micro-Laser Therapy and Feeder Vessel Treatment), Proton Beam Therapy, microstimulation therapy, Retinal Detachment and Vitreous Surgery, Scleral Buckle, Submacular Surgery, Transpupillary Thermotherapy, Photosystem I therapy, use of RNA interference (RNAi), extracorporeal rheopheresis (also known as membrane differential filtration and Rheotherapy), microchip implantation, stem cell therapy, gene replacement therapy, ribozyme gene therapy (including gene therapy for hypoxia response element, Oxford

Biomedica; Lentipak, Genetix; PDEF gene therapy, GenVec), photoreceptor/retinal cells transplantation (including transplantable retinal epithelial cells, Diacrin, Inc.; retinal cell transplant, Cell Genesys, Inc.), and acupuncture.

Any anti-angiogenic agent can be used in combination with the bispecific antibody or pharmaceutical composition according to the invention, including, bu not limited to, those listed by Carmeliet and Jain, 2000, Nature 407:249-257. In certain embodiments, the anti-angiogenic agent is another VEGF antagonist or a VEGF receptor antagonist such as VEGF variants, soluble VEGF receptor fragments, aptamers capable of blocking VEGF or VEGFR, neutralizing anti- VEGFR antibodies, low molecule weight inhibitors of VEGFR tyrosine kinases and any combinations thereof and these include anti- VEGF aptamers (e.g. Pegaptanib), soluble recombinant decoy receptors (e.g. VEGF Trap). . In certain embodiments, the anti-angiogenic agent is include corticosteroids, angiostatic steroids, anecortave acetate, angiostatin, endostatin, small interfering RNA's decreasing expression of VEGFR or VEGF ligand, post-VEGFR blockade with tyrosine kinase inhibitors, MMP inhibitors, IGFBP3, SDF-1 blockers, PEDF, gamma-secretase, Delta-like ligand 4, integrin antagonists, HIF-1 alpha blockade, protein kinase CK2 blockade, and inhibition of stem cell (i.e. endothelial progenitor cell) homing to the site of neovascularization using vascular endothelial cadherin (CD-144) and stromal derived factor (SDF)-I antibodies. Small molecule RTK inhibitors targeting VEGF receptors including PTK787 can also be used. Agents that have activity against neovascularization that are not necessarily anti-VEGF compounds can also be used and include anti-inflammatory drugs, m-Tor inhibitors, rapamycin, everolismus, temsirolismus, cyclospohne, anti-TNF agents, anti-complement agents, and nonsteroidal antiinflammatory agents. Agents that are neuroprotective and can potentially reduce the progression of dry macular degeneration can also be used, such as the class of drugs called the 'neurosteroids.' These include drugs such as dehydroepiandrosterone(DHEA)(Brand names: Prastera(R) and Fidelin(R)), dehydroepiandrosterone sulfate, and pregnenolone sulfate. Any AMD (age-related macular degeneration) therapeutic agent can be used in combination with the bispecific antibody or pharmaceutical composition according to the invention, including but not limited to verteporfin in combination with PDT, pegaptanib sodium, zinc, or an antioxidant(s), alone or in any combination.

The terms "subject" and "patient" are used interchangeably and refer to mammals such as human patients and non-human primates, as well as experimental animals such as rabbits, rats, and mice, and other animals. Animals include all vertebrates, e.g., mammals and non-mammals, such as dogs, cats, sheeps, cows, pigs, rabbits, chickens, and etc. Preferred subjects for practicing the therapeutic methods of the present invention are human. Subjects in need of treatment include patients already suffering from an ocular vascular disease or disorder as well as those prone to developing the disorder.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

The term “transformation” as used herein refers to process of transfer of a vectors/nucleic acid into a host cell. If cells without formidable cell wall barriers are used as host cells, transfection is carried out e.g. by the calcium phosphate precipitation method as described by Graham, F.L., van der Eb, A.J., Virology 52 (1973) 546-467. However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used. If prokaryotic cells or cells which contain substantial cell wall constructions are used, e.g. one method of transfection is calcium treatment using calcium chloride as described by Cohen, S.N., et al., PNAS. 69 (1972) 2110-2114.

As used herein, "expression" refers to the process by which a nucleic acid is transcribed into mRNA and/or to the process by which the transcribed mRNA (also referred to as transcript) is subsequently being translated into peptides, polypeptides, or proteins. The transcripts and the encoded polypeptides are collectively referred to as gene product. If the polynucleotide is derived from genomic DNA, expression in a eukaryotic cell may include splicing of the mRNA.

A "vector" is a nucleic acid molecule, in particular self-replicating, which transfers an inserted nucleic acid molecule into and/or between host cells. The term includes vectors that function primarily for insertion of DNA or RNA into a cell (e.g., chromosomal integration), replication of vectors that function primarily for the replication of DNA or RNA, and expression vectors that function for transcription and/or translation of the DNA or RNA. Also included are vectors that provide more than one of the functions as described.

An "expression vector" is a polynucleotide which, when introduced into an appropriate host cell, can be transcribed and translated into a polypeptide. An “expression system" usually refers to a suitable host cell comprised of an expression vector that can function to yield a desired expression product.

The following examples, sequence listing and figures are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

## Description of the Sequence Listing (Amino acid sequences)

| | | |
|---|---|---|
| SEQ ID NO: | 1 | heavy chain CDR3H, <VEGF>ranibizumab |
| SEQ ID NO: | 2 | heavy chain CDR2H, <VEGF>ranibizumab |
| SEQ ID NO: | 3 | heavy chain CDR1H, <VEGF>ranibizumab |
| SEQ ID NO: | 4 | light chain CDR3L, <VEGF>ranibizumab |
| SEQ ID NO: | 5 | light chain CDR2L, <VEGF>ranibizumab |
| SEQ ID NO: | 6 | light chain CDR1L, <VEGF>ranibizumab |
| SEQ ID NO: | 7 | heavy chain variable domain VH, <VEGF>ranibizumab |
| SEQ ID NO: | 8 | light chain variable domain VL, <VEGF>ranibizumab |
| SEQ ID NO: | 9 | heavy chain CDR3H, <ANG-2> Ang2i_LC10 variant |
| SEQ ID NO: | 10 | heavy chain CDR2H, <ANG-2> Ang2i_LC10 variant |
| SEQ ID NO: | 11 | heavy chain CDR1H, <ANG-2> Ang2i_LC10 variant |
| SEQ ID NO: | 12 | light chain CDR3L, <ANG-2> Ang2i_LC10 variant |

| | | |
|---|---|---|
| SEQ ID NO: | 13 | light chain CDR2L, <ANG-2> Ang2i_LC10 variant |
| SEQ ID NO: | 14 | light chain CDR1L, <ANG-2> Ang2i_LC10 variant |
| SEQ ID NO: | 15 | heavy chain variable domain VH, <ANG-2> Ang2i_LC10 variant |
| SEQ ID NO: | 16 | light chain variable domain VL, <ANG-2> Ang2i_LC10 variant |
| SEQ ID NO: | 17 | Human vascular endothelial growth factor (VEGF); precursor sequence of human VEGF165 |
| SEQ ID NO: | 18 | Human angiopoietin-2 (ANG-2) |
| SEQ ID NO: | 19 | Human angiopoietin-1 (ANG-1) |
| SEQ ID NO: | 20 | Human Tie-2 receptor |
| SEQ ID NO | 21 | Heavy chain 1 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations (VEGFang2-0012) |
| SEQ ID NO | 22 | Heavy chain 2 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations (VEGFang2-0012) |
| SEQ ID NO | 23 | Light chain 1 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations (VEGFang2-0012) |
| SEQ ID NO | 24 | Light chain 2 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations (VEGF-Ang2-0012) |
| SEQ ID NO: | 25 | Heavy chain 1 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations and P329G LALA mutations (VEGFang2-0016) |
| SEQ ID NO: | 26 | Heavy chain 2 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations and P329G LALA mutations (VEGFang2-0016) |
| SEQ ID NO: | 27 | Light chain 1 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations and P329G LALA mutations (VEGFang2-0016) |
| SEQ ID NO: | 28 | Light chain 2 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations and P329G LALA mutations (VEGFang2-0016) |
| SEQ ID NO: | 29 | Heavy chain 1 of <VEGF-ANG-2> CrossMAb IgG4 with AAA mutations and with SPLE mutations |
| SEQ ID NO: | 30 | Heavy chain 2 of <VEGF-ANG-2> CrossMAb IgG4 with AAA mutations and with SPLE mutations |

| | | |
|---|---|---|
| SEQ ID NO: | 31 | Light chain 1 of <VEGF-ANG-2> CrossMAb IgG4 with AAA mutations and with SPLE mutations |
| SEQ ID NO: | 32 | Light chain 2 of <VEGF-ANG-2> CrossMAb IgG4 with AAA mutations and with SPLE mutations |
| SEQ ID NO: | 33 | Heavy chain 1 of <VEGF-ANG-2> OAscFab IgG1 with AAA mutations |
| SEQ ID NO: | 34 | Heavy chain 2 of <VEGF-ANG-2> OAscFab IgG1 with AAA mutations |
| SEQ ID NO: | 35 | Light chain 1 of <VEGF-ANG-2> OAscFab IgG1 with AAA mutations |
| SEQ ID NO: | 36 | Heavy chain 1 of <VEGF-ANG-2> OAscFab IgG4 with AAA mutations and with SPLE mutations |
| SEQ ID NO: | 37 | Heavy chain 2 of <VEGF-ANG-2> OAscFab IgG4 with AAA mutations and with SPLE mutations |
| SEQ ID NO: | 38 | Light chain 1 of <VEGF-ANG-2> OAscFab IgG4 with AAA mutations and with SPLE mutations |
| SEQ ID NO: | 39 | Heavy chain 1 of <VEGF-ANG-2> CrossMAb IgG1 wild type (without AAA mutations) (VEGFang2-0201) |
| SEQ ID NO: | 40 | Heavy chain 2 of <VEGF-ANG-2> CrossMAb IgG1 wild type (without AAA mutations) (VEGFang2-0201) |
| SEQ ID NO: | 41 | Light chain 1 of <VEGF-ANG-2> CrossMAb IgG1 wild type ( without AAA mutations) (VEGFang2-0201) |
| SEQ ID NO: | 42 | Light chain 2 of <VEGF-ANG-2> CrossMAb IgG1 wild type (without AAA mutations) (VEGFang2-0201) |
| SEQ ID NO: | 43 | Heavy chain 1 of <VEGF-ANG-2> CrossMAb IgG1 with P329G LALA mutations only (without AAA mutations) (VEGFang2-0015) |
| SEQ ID NO: | 44 | Heavy chain 2 of <VEGF-ANG-2> CrossMAb IgG1 with P329G LALA mutations only (without AAA mutations) (VEGFang2-0015) |
| SEQ ID NO: | 45 | Light chain 1 of <VEGF-ANG-2> CrossMAb IgG1 with P329G LALA mutations only (without AAA mutations) (VEGFang2-0015) |
| SEQ ID NO: | 46 | Light chain 2 of <VEGF-ANG-2> CrossMAb IgG1 with P329G LALA mutations only (without AAA mutations) (VEGFang2-0015) |
| SEQ ID NO: | 47 | kappa light chain constant region |
| SEQ ID NO: | 48 | lambda light chain constant region |

| | | |
|---|---|---|
| SEQ ID NO: | 49 | heavy chain constant region derived from human IgG1 |
| SEQ ID NO: | 50 | heavy chain constant region derived from human IgG4 |

**In the following, embodiments of the invention are listed:**

1. 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)

2. The bispecific antibody according to embodiment 1, 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.

3. The bispecific antibody according to any one of embodiments 1 to 2, wherein the constant heavy chain region under iii) is of IgG1 subclass

4. The bispecific antibody according to embodiment 3, wherein the constant heavy chain region of IgG1 subclass further comprises the mutations L234A , L235A and P329G (numbering according to EU Index of Kabat)

5. The bispecific antibody according to any one of embodiments 1 to 2, wherein the constant heavy chain region under iii) is of IgG4 subclass

6. The bispecific antibody according to embodiment 5, wherein the constant heavy chain region of IgG4 subclass further comprises the mutations S228P and L235E (numbering according to EU Index of Kabat)

7. The bispecific antibody according to embodiment 5, wherein the constant heavy chain region of IgG4 subclass further comprises the mutations S228P , L235E and P329G (numbering according to EU Index of Kabat)

8 A pharmaceutical composition comprising an antibody according to any one of embodiments 1 to 7.

9. The bispecific antibody according to any one of embodiments 1 to 7 for use in the treatment of ocular vascular diseases.

10. Use of the bispecific antibody according to any one of embodiments 1 to 7 for the manufacture of a medicament for the treatment of ocular vascular diseases.

11. The bispecific antibody according to any one of embodiments 9 or 10, wherein the antibody is administered via intravitreal application.

12. A method of treatment of patient suffering from ocular vascular diseases by administering an antibody according to any one of embodiments 1 to 7 to a patient in the need of such treatment.

13. A nucleic acid encoding a bispecific antibody according to any one of embodiments 1 to 7.

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

15. A prokaryotic or eukaryotic host cell comprising a vector according to embodiment 14.

16. A method for the preparation of a bispecific antibody according to embodiments 1 to 7

    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.

17. A bispecific antibody obtained by the method of embodiment 16.

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.

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.

20. 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.

## Experimental procedures

**Table 1: Bispecific antibodies and their respective sequences**

| Description | Short Name | Sequences |
|---|---|---|
| <VEGF-ANG-2> CrossMAb IgG1 **with AAA** mutations | VEGFang2-0012 | SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24 |
| <VEGF-ANG-2> CrossMAb IgG1 wild type (**without** AAA mutations) | VEGFang2-0201- | SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42 |
| <VEGF-ANG-2> CrossMAb IgG1 **with AAA** mutations and P329G LALA mutations | VEGFang2-0016 | SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 |
| <VEGF-ANG-2> CrossMAb IgG1 with P329G LALA mutations only (**without** AAA mutations) | VEGFang2-0015 | SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46 |
| <VEGF-ANG-2> CrossMAb IgG4 with AAA mutations and with SPLE mutations | -- | SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32 |
| <VEGF-ANG-2> OAscFab IgG1 with AAA mutations | - | SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35 |
| <VEGF-ANG-2> OAscFab IgG4 with AAA mutations and with SPLE mutations | - | SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38 |

Please note that the term “with (the) mutations AAA” as used herein refers the mutations I253A (Ile253Ala), H310A (His310Ala), and H435A (His435Ala) in the constant heavy chain region of IgG1 or IgG4 (numbering according to EU Index of Kabat), the term “with (the) mutations P329G LALA” as used herein refers to the mutations L234A (Leu235Ala) , L235A (Leu234Ala) and P329G (Pro329Gly) in the constant heavy chain region of IgG1 subclass (numbering according to EU Index of Kabat), and the term “with (the) mutations SPLE” as used herein refers to the S228P (Ser228Pro) and L235E (Leu235Glu) the constant heavy chain region of IgG4 subclass (numbering according to EU Index of Kabat).

## Examples

### Materials & general methods

General information regarding the nucleotide sequences of human immunoglobulin light and heavy chains is given in: Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991). Amino acids of antibody chains are numbered and referred to according to EU numbering (Edelman, G.M., et al., Proc. Natl. Acad. Sci. USA 63 (1969) 78-85; Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)).

### Recombinant DNA techniques

Standard methods were used to manipulate DNA as described in Sambrook, J. et al., Molecular Cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). The molecular biological reagents were used according to the manufacturer's instructions.

### Gene synthesis

Desired gene segments were ordered according to given specifications at Geneart (Regensburg, Germany).

### DNA sequence determination

DNA sequences were determined by double strand sequencing performed at MediGenomix GmbH (Martinsried, Germany) or Sequiserve GmbH (Vaterstetten, Germany).

### DNA and protein sequence analysis and sequence data management

The GCG's (Genetics Computer Group, Madison, Wisconsin) software package version 10.2 and Infomax's Vector NT1 Advance suite version 8.0 was used for sequence creation, mapping, analysis, annotation and illustration.

## Expression vectors

For the expression of the described antibodies, variants of expression plasmids for transient expression (e.g. in HEK293-F) cells based either on a cDNA organization with or without a CMV-Intron A promoter or on a genomic organization with a CMV promoter were applied.

Beside the antibody expression cassette the vectors contained:

- an origin of replication which allows replication of this plasmid in *E. coli*,
- a ß-lactamase gene which confers ampicillin resistance in *E. coli.*, and
- the dihydrofolate reductase gene from *Mus musculus* as a selectable marker in eukaryotic cells
- The transcription unit of the antibody gene was composed of the following elements:
- unique restriction site(s) at the 5' end
- the immediate early enhancer and promoter from the human cytomegalovirus,
- followed by the Intron A sequence in the case of the cDNA organization,
- a 5'-untranslated region of a human antibody gene,
- an immunoglobulin heavy chain signal sequence,
- the human antibody chain (wildtype or with domain exchange) either as cDNA or as genomic organization with the immunoglobulin exon-intron organization
- a 3' untranslated region with a polyadenylation signal sequence, and
- unique restriction site(s) at the 3' end.

The fusion genes comprising the antibody chains as described below were generated by PCR and/or gene synthesis and assembled by known recombinant methods and techniques by connection of the according nucleic acid segments *e.g.* using unique restriction sites in the respective vectors. The subcloned nucleic acid sequences were verified by DNA sequencing. For transient transfections larger quantities of the plasmids were prepared by plasmid preparation from transformed *E. coli* cultures (Nucleobond AX, Macherey-Nagel).

## Cell culture techniques

Standard cell culture techniques were used as described in Current Protocols in Cell Biology (2000), Bonifacino, J.S., Dasso, M., Harford, J.B., Lippincott-Schwartz, J. and Yamada, K.M. (eds.), John Wiley & Sons, Inc.

The bispecific antibodies were expressed by transient co-transfection of the respective expression plasmids in in HEK29-F cells growing in suspension as described below.

## Example 1

## Expression and Purification

### Transient transfections in HEK293-F system

The bispecific antibodies were generated by transient transfection with the respective plasmids (e.g. encoding the heavy and modified heavy chain, as well as the corresponding light and modified light chain) using the HEK293-F system (Invitrogen) according to the manufacturer's instruction. Briefly, HEK293-F cells (Invitrogen) growing in suspension either in a shake flask or in a stirred fermenter in serum-free FreeStyle™ 293 expression medium (Invitrogen) were transfected with a mix of the four expression plasmids and 293fectin™ or fectin (Invitrogen). For 2 L shake flask (Corning) HEK293-F cells were seeded at a density of 1.0E*6 cells/mL in 600 mL and incubated at 120 rpm, 8% CO2. The day after the cells were transfected at a cell density of ca. 1.5E*6 cells/mL with ca. 42 mL mix of A) 20 mL Opti-MEM (Invitrogen) with 600 µg total plasmid DNA (1 µg/mL) encoding the heavy or modified heavy chain, respectively and the corresponding light chain in an equimolar ratio and B) 20 ml Opti-MEM + 1.2 mL 293 fectin or fectin (2 µl/mL). According to the glucose consumption glucose solution was added during the course of the fermentation. The supernatant containing the secreted antibody was harvested after 5-10 days and antibodies were either directly purified from the supernatant or the supernatant was frozen and stored.

### Purification

Bispecific antibodies were purified from cell culture supernatants by affinity chromatography using MabSelectSure-Sepharose™ (for non_AAA mutants) (GE Healthcare, Sweden) or kappaSelect-Agarose (for AAA mutants) (GE Healthcare, Sweden), hydrophobic interaction chromatography using butyl-Sepharose (GE Healthcare, Sweden) and Superdex 200 size exclusion (GE Healthcare, Sweden) chromatography.

Briefly, sterile filtered cell culture supernatants were captured on a MabSelect SuRe resin equilibrated with PBS buffer (10 mM $Na_2HPO_4$, 1 mM $KH_2PO_4$, 137 mM NaCl and 2.7 mM KCl, pH 7.4), washed with equilibration buffer and eluted with 25 mM sodium citrate at pH 3.0. The AAA mutants were captured on a kappaSelect resin equilibrated with 25 mM Tris, 50 mM NaCl, pH 7.2, washed with equilibration buffer and eluted with 25 mM sodium citrate pH 2.9. The eluted protein fractions were pooled and neutralized with 2M Tris, pH 9.0. The antibody pools were prepared for hydrophobic interaction chromatography by adding 1.6 M ammonium sulfate solution to a final concentration of 0.8 M ammonium sulfate and the pH adjusted to pH 5.0 using acetic acid. After equilibration of the butyl-Sepharose resin with 35 mM sodium acetate, 0.8 M ammonium sulfate, pH 5.0, the antibodies were applied to the resin, washed with equilibration buffer and eluted with a linear gradient to 35 mM sodium acetate pH 5.0. The bispecific antibody containing fractions were pooled and further purified by size exclusion chromatography using a Superdex 200 26/60 GL (GE Healthcare, Sweden) column equilibrated with 20 mM histidine, 140 mM NaCl, pH 6.0. The bispecific antibody containing fractions were pooled, concentrated to the required concentration using Vivaspin ultrafiltration devices (Sartorius Stedim Biotech S.A., France) and stored at -80°C.

**Table 2: Yields of bispecific <VEGF-ANG-2> antibodies**

| | **VEGFang2-0015 (without AAA mutation )** | **VEGFang2-0016 (with AAA mutation)** |
|---|---|---|
| Titer supernatant | 64 µg/ml, (2 L = 128 mg) | n.a. (2 L scale) |
| Protein A (MabSelectSure) | 118 mg (~ 70% monomer) | n.a. |
| Kappa Select | n.a. | 117 mg (~ 83% monomer) |
| Butyl Sepharose | 60 mg | 57 mg |
| SEC | 35 mg (>95% monomer) | 38 mg (>95% monomer) |

Purity and antibody integrity were analyzed after each purification step by CE-SDS using microfluidic Labchip technology (Caliper Life Science, USA). 5 µl of protein solution was prepared for CE-SDS analysis using the HT Protein Express Reagent Kit according manufacturer's instructions and analysed on LabChip GXII system using a HT Protein Express Chip. Data were analyzed using LabChip GX Software.

**Table 3: Removal of typical side products by different sequential purification steps determined by CE-SDS.**

| Purifi-cation Step | VEGFang2-0015 | | | | | | VEGFang2-0016 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % peak area* * analysis: CE-SDS (Caliper Labchip GXII) | | | | | | | | | | | |
| | mab | ¾ ab | (HC)2 | ½ ab | (LC)2 | LC | mab | ¾ ab | (HC)2 | ½ ab | (LC) 2 | LC |
| Mab Select | 55,7 | 19 | 10,6 | 9,8 | 3,5 | 0,9 | - | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sure | | | | | | | | | | | | |
| Kappa Select | - | | | | | | 63 | 13,4 | 3,5 | 6,1 | 5,8 | 7,4 |
| Butyl-Sepharose | 81,4 | 1,9 | 2,3 | 8,2 | 3,6 | 1,8 | 76,2 | 1,3 | 0,7 | 8,3 | 7,7 | 5,8 |
| Superdex 200_SEC | 92,4 | 1,8 | 2,6 | 1,4 | 0,5 | 0,5 | 99 | 1,1 | n.d. | n.d. | n.d. | n.d. |

The aggregate content of antibody samples was analyzed by high-performance SEC using a Superdex 200 analytical size-exclusion column (GE Healthcare, Sweden) in 2xPBS (20 mM $Na_2HPO_4$, 2 mM $KH_2PO_4$, 274 mM NaCl and 5.4 mM KCl, pH 7.4) running buffer at 25°C. 25 µg protein were injected on the column at a flow rate of 0.75 ml/min and eluted isocratic over 50 minutes.

Analogously the <VEGF-ANG-2> bispecific antibodies VEGFang2-0012 and VEGFang2-0201 were prepared and purified with the following yields:

| | VEGFang2-0012 (with AAA mutation ) | VEGFang2-0201 (without AAA mutation) |
|---|---|---|
| Titer //amount | - | 36 µg/ml // 72 mg |
| Scale | 2,1 L | 2L |
| Protein A (MabSelectSure) | - | 66 mg (~95 % monomer) |
| kappaSelect | 43 mg(~ 65 % monomer) | - |
| Butyl Sepharose | - | 45 mg |
| SEC | 14 mg | 21 mg (> 98 % monomer) |
| Yield hydoxylapatite | 8,5 mg (> 98% monomer) | |
| Totatl yield (recovery) | 8,5 mg (20%) | 21 mg (30%) |

Also the <VEGF-ANG-2> bispecific antibodies <VEGF-ANG-2> CrossMAb IgG4 with AAA mutations and with SPLE mutations(SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32), <VEGF-ANG-2> OAscFab IgG1 with AAA mutations(SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35)and <VEGF-ANG-2> OAscFab IgG4 with AAA mutations and with SPLE mutations(SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38) can be prepared and purified analogously.

## Example 2

## Analytics & Developability

### Small-scale DLS-based viscosity measurement.

Viscosity measurement was essentially performed as described in (He, F. et al., Analytical Biochemistry 399 (2009) 141-3). Briefly, samples are concentrated to various protein concentrations in 200 mM arginine succinate, pH 5.5, before polystyrene latex beads (300 nm diameter) and Polysorbate 20 (0.02% v/v) are added. Samples are transferred into an optical 384-well plate by centrifugation through a 0.4 µm filter plate and covered with paraffine oil. The apparent diameter of the latex beads is determined by dynamic light scattering at 25 °C. The viscosity of the solution can be calculated as η = η0(rh/rh,0) (η: viscosity; η0: viscosity of water; rh: apparent hydrodynamic radius of the latex beads; rh,0: hydrodynamic radius of the latex beads in water.

To allow comparison of various samples at the same concentration, viscosity-concentration data were fitted with the Mooney equation (Equation 1) (Mooney, Colloid Sci, 1951; Monkos, Biochem. Biophys. Acta 1997) and data interpolated accordingly.

$$\eta = \eta_0 \exp\left(\frac{S\Phi}{1-K\Phi}\right)$$ **Equation 1**

(S: hydrodynamic interaction parameter of the protein; K: self-crowding factor; Φ: volume fraction of the dissolved protein)

Results are shown in Figure 2: VEGFang2-0016 with AAA mutations in the Fc part shows a lower viscosity at all measured temperatures compared to VEGFang2-0015 without the AAA mutations in the Fc part.

### DLS aggregation onset temperature

Samples are prepared at a concentration of 1 mg/mL in 20 mM Histidine/Histidine chloride, 140 mM NaCl, pH 6.0, transferred into an optical 384-well plate by centrifugation through a 0.4 µm filter plate and covered with paraffine oil. The hydrodynamic radius is measured repeatedly by dynamic light scattering while the samples are heated with a rate of 0.05 °C/min from 25 °C to 80 °C. The aggregation onset temperature is defined as the temperature at which the hydrodynamic radius starts to increase. Results are shown in Figure 3. In Figure 3 the aggregation of VEGFang2-0015 without the AAA mutations versus VEGFang2-0016 with AAA mutations in the Fc part is shown. VEGFang2-0016 showed a aggregation onset temperature of 61 °C whereas VEGFang2-0015 without the AAA mutations showed a onset temperature of 60 °C.

**DLS timecourse**

Samples are prepared at a concentration of 1 mg/mL in 20 mM Histidine/Histidine chloride, 140 mM NaCl, pH 6.0, transferred into an optical 384-well plate by centrifugation through a 0.4 μm filter plate and covered with paraffine oil. The hydrodynamic radius is measured repeatedly by dynamic light scattering while the samples are kept at a constant temperature of 50°C for up to 145 hours. In this experiment, aggregation tendencies of the native, unfolded protein at elevated temperature would lead to an increase of the average particle diameter over time. This DLS-based method is very sensitive for aggregates because these contribute over-proportionally to the scattered light intensity. Even after 145 hours at 50 °C (a temperature close to the aggregation-onset temperature, see above), an average particle diameter increase of only less than 0.5 nm was found for both VEGFang2-0015 and VEGFang2-0016

**7 day storage at 40°C at 100 mg/ml (HMW increase)**

Samples are concentrated to a final concentration of 100 mg/mL in 200 mM arginine succinate, pH 5.5, sterile filtered and quiescently stored at 40°C for 7 days. Before and after storage, the content of high and low molecular weight species (HMWs and LMWs, respectively) is determined by size-exclusion chromatography. The difference in HMW and LMW content between the stored sample and a sample measured immediately after preparation is reported as “HMW increase” and “LMW increase”, respectively. Results are shown in Table 4 and Figure 4, which show that VEGFang2-0015 (without AAA mutation ) shows a higher reduction of the main peak and a higher HMW increase compared to VEGF Ang2-0016 (with AAA mutation). Surprisingly VEGF Ang2-0016 (with AAA mutation) showed a lower aggregation tendency compared toVEGFang2-0015 (without AAA mutation).

**Table 4: Delta Main-, HMW and LMW peaks after 7d at 40 °C**

| | delta_area%(40°C-(-80°C)) | | |
|---|---|---|---|
| | Main Peak | HMW | LMW |
| VEGFang2-0015 (-AAA mutations) | -3,56 | 2,89 | 0,67 |
| VEGFang2-0016 (+AAA mutations) | -1,74 | 1,49 | 0,25 |

The functional analysis of anti-VEGF and anti-Ang2 bispecific antibodies was assessed by Surface Plasmon Resonance (SPR) using a BIAcore® T100 or T200 instrument (GE Healthcare) at 25°C. The BIAcore® system is well established for the study of molecule interactions. SPR-technology is based on the measurement of the refractive index close to the surface of a gold coated biosensor chip. Changes in the refractive index indicate mass changes on the surface caused by the interaction of immobilized ligand with analyte injected in solution. The mass increases if molecules bind immobilized ligands on the surface, and vice versa, the mass decreases in case of dissociation of the analyte from the immobilized ligand (reflecting complex dissociation). SPR allows a continuous real-time monitoring of ligand/analyte binding and thus the determination of the association rate constant (ka), the dissociation rate constant (kd), and of the equilibrium constant (KD).

## Example 3

## Binding to VEGF, Ang2, FcgammaR and FcRn

### VEGF isoforms kinetic affinity including assessment of species-crossreactivity

Around 12000 resonance units (RU) of the capturing system (10 µg/ml goat anti human F(ab)’$_2$; Order Code: 28958325; GE Healthcare Bio-Sciences AB, Sweden) were coupled on a CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. The sample and system buffer was PBS-T (10 mM phosphate buffered saline including 0.05% Tween20) pH 7.4. The flow cell was set to 25 °C - and the sample block set to 12 °C - and primed with running buffer twice. The bispecific antibody was captured by injecting a 50 nM solution for 30 sec at a flow of 5 µl/min. Association was measured by injection of human hVEGF121, mouse mVEGF120 or rat rVEGF164 in various concentrations in solution for 300 sec at a flow of 30 µl/min starting with 300 nM in 1:3 dilutions. The dissociation phase was monitored for up to 1200 sec and triggered by switching from the sample solution to running buffer. The surface was regenerated by 60 sec washing with a Glycine pH 2.1 solution at a flow rate of 30 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from a goat anti human $F(ab')_2$ surface. Blank injections are also subtracted (= double referencing). For calculation of apparent $K_D$ and other kinetic parameters the Langmuir 1:1 model was used. Results are shown in Table 5.

**Ang2 solution affinity including assessment of species-crossreactivity**

Solution affinity measures the affinity of an interaction by determining the concentration of free interaction partners in an equilibrium mixture. The solution affinity assay involves the mixing of an <VEGF-ANG-2> bispecific antibody, kept at a constant concentration, with a ligand (= Ang2) at varying concentrations. Maximum possible resonance units (e.g. 17000 resonance units (RU)) of an antibody was immobilized on the CM5 chip (GE Healthcare BR-1005-30) surface at pH 5.0 using an amine coupling kit supplied by the GE Healthcare. The sample and system buffer was HBS-P pH 7.4. Flow cell was set to 25 °C and sample block to 12 °C and primed with running buffer twice. To generate a calibration curve increasing concentrations of Ang2 were injected into a BIAcore flowcell containing the immobilized VEGF-ANG-2> bispecific antibody. The amount of bound Ang2 was determined as resonance units (RU) and plotted against the concentration. Solutions of each ligand (11 concentrations from 0 to 200 nM for the VEGF-ANG-2> bispecific antibody) were incubated with 10 nM Ang2 and allowed to reach equilibrium at room temperature. Free Ang2 concentrations were determined from calibration curve generated before and after measuring the response of solutions with known amounts of Ang2. A 4-parameter fit was set with XLfit4 (IDBS Software) using Model 201 using free Ang2 concentration as y-axis and used concentration of antibody for inhibition as x-axis. The affinity was calculated by determining the inflection point of this curve. The surface was regenerated by one time 30 sec washing with a 0.85% $H_3PO_4$ solution at a flow rate of 30 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from a blank-coupled surface. Results are shown in Table 6.

**FcRn steady state affinity**

For FcRn measurement a steady state affinity was used to compare bispecific antibodies against each other. Human FcRn was diluted into coupling buffer (10 µg/ml, Na-Acetate pH5.0) and immobilized on a C1-Chip (GE Healthcare BR-

1005-35) by targeted immobilization procedure using a BIAcore wizard to a final response of 200 RU. Flow cell was set to 25 °C and sample block to 12 °C and primed with running buffer twice. The sample and system buffer was PBS-T (10 mM phosphate buffered saline including 0.05% Tween20) pH 6.0. To assess different IgG concentrations for each antibody, a concentration of 62.5 nM, 125 nM and 250 nM, 500 nM was prepared. Flow rate was set to 30 µl/min and the different samples were injected consecutively onto the chip surface choosing 180 sec association time. The surface was regenerated by injected PBS-T pH 8 for 60 sec at a flow rate of 30 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from a blank surface. Buffer injections are also subtracted (= double referencing). For calculation of steady state affinity the method from the Bia-Evaluation software was used. Briefly, the RU values (RU max) were plotted against the analysed concentrations, yielding a dose-response curve. Based on a 2-parametric fit, the upper asymptote is calculated, allowing the determination of the half-maximal RU value and hence the affinity. Results are shown in Figure 5 and Table 7. Analogously the affinity to cyno, mouse and rabbit FcRn can be determined.

### FcgammaRIIIa measurement

For FcgammaRIIIa measurement a direct binding assay was used. Around 3000 resonance units (RU) of the capturing system (1 µg/ml Penta-His; Quiagen) were coupled on a CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. The sample and system buffer was HBS-P+ pH 7.4. The flow cell was set to 25 °C - and sample block to 12 °C - and primed with running buffer twice. The FcgammaRIIIa -His-receptor was captured by injecting a 100 nM solution for 60 sec at a flow of 5 µl/min. Binding was measured by injection of 100 nM of bispecific antibody or monospecific control antibodies (anti-Dig for IgG1 subclass and an IgG4 subclass antibody) for 180 sec at a flow of 30 µl/. The surface was regenerated by 120 sec washing with Glycine pH 2.5 solution at a flow rate of 30 µl/min. Because FcgammaRIIIa binding differs from the Langmuir 1:1 model, only binding/no binding was determined with this assay. In a similar manner FcgammaRIa , and FcgammaRIIa binding can be determined. Results are shown in Figure 6, where it follows that by introduction of the mutations P329G LALA no more binding to FcgammaRIIIa could be detected.

## Assessment of independent VEGF- and Ang2-binding to the <VEGF-ANG-2> bispecific antibodies

Around 3500 resonance units (RU) of the capturing system (10 µg/ml goat anti human IgG; GE Healthcare Bio-Sciences AB, Sweden) were coupled on a CM4 chip (GE Healthcare BR-1005-34) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. The sample and system buffer was PBS-T (10 mM phosphate buffered saline including 0.05% Tween20 ) pH 7.4. The temperature of the flow cell was set to 25 °C and of the sample block to 12 °C. Before capturing, the flow cell was primed with running buffer twice.

The bispecific antibody was captured by injecting a 10 nM solution for 60 sec at a flow of 5 µl/min. Independent binding of each ligand to the bispecific antibody was analysed by determining the active binding capacity for each ligand, either added sequentially or simultaneously (flow of 30 µl/min):

1. Injection of human VEGF with a concentration of 200 nM for 180 sec (identifies the single binding of the antigen).
2. Injection of human Ang2 with a concentration of 100 nM for 180 sec (identifies single binding of the antigen).
3. Injection of human VEGF with a concentration of 200 nM for 180 sec followed by an additional injection of human Ang2 with a concentration of 100 nM for 180 sec (identifies binding of Ang2 in the presence of VEGF).
4. Injection of human Ang2 with a concentration of 100 nM for 180 sec followed by an additional injection of human VEGF with a concentration of 200 nM (identifies binding of VEGF in the presence of Ang2).
5. Co-Injection of human VEGF with a concentration of 200 nM and of human Ang2 with a concentration of 100 nM for 180 sec (identifies the binding of VEGF and of Ang2 at the same time).

The surface was regenerated by 60 sec washing with a 3m MgCl2 solution at a flow rate of 30 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from a goat anti human IgG surface.

The bispecific antibody is able to bind both antigens mutual independently if the resulting final signal of the approaches 3, 4 & 5 equals or is similar to the sum of the individual final signals of the approaches 1 and 2. Results are shown in Table 9, where both antibodies VEGFang2-0016, VEGFang2-0012 are shown to be able to bind mutual independently to VEGF and ANG2

**Assessment of simultaneous VEGF- and Ang2-binding to the <VEGF-ANG-2> bispecific antibodies**

First, around 1600 resonance units (RU) of VEGF (20µg/ml) were coupled on a CM4 chip (GE Healthcare BR-1005-34) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. The sample and system buffer was PBS-T (10 mM phosphate buffered saline including 0.05% Tween 20) pH 7.4. Flow cell was set to 25 °C and sample block to 12 °C and primed with running buffer twice. Second, 50nM solution of the bispecific antibody was injected for 180 sec at a flow of 30 µl/min. Third, hAng-2 was injected for 180 sec at a flow of 30 µl/min. The binding response of hAng-2 depends from the amount of the bispecific antibody bound to VEGF and shows simultaneous binding. The surface was regenerated by 60 sec washing with a 0.85% H3PO4 solution at a flow rate of 30 µl/min. Simultaneous binding is shown by an additional specific binding signal of hAng2 to the previous VEGF bound <VEGF-ANG-2> bispecific antibodies. For both bispecific antibodies VEGFang2-0015 and VEGFang2-0016 simultaneous VEGF- and Ang2-binding to the <VEGF-ANG-2> bispecific antibodies could be detected (data not shown).

**Table 5: Results: Kinetic affinities to VEGF isoforms from different species**

| | **VEGFang2-0015 - apparent affinity** | **VEGFang2-0016 - apparent affinity** | **VEGFang2-0012 - apparent affinity** | **VEGFang2-0201 - apparent affinity** |
|---|---|---|---|---|
| Human VEGF 121 | ≤1 pM (out of Biacore specification) | ≤1 pM (out of Biacore specification) | ≤1 pM (out of Biacore specification) | ≤1 pM (out of Biacore specification) |
| mouseVEGF 120 | no binding | no binding | no binding | no binding |
| Rat VEGF 164 | 13 nM | 14 nM | 24 nM | 35 nM |

**Table 6: Results: Solution affinities to Ang2**

| | VEGFang2-0015 KD [nM] | VEGFang2-0016 KD [nM] | VEGFang2-0012 KD [nM] | VEGFang2--0201 KD [nM] |
|---|---|---|---|---|
| humanAng2 | 8 | 20 | 20 | tbd |
| cynoAng2 | 5 | 13 | 10 | tbd |
| mouseAng2 | 8 | 13 | 8 | tbd |
| rabbitAng2 | 4 | 11 | 8 | tbd |

**Table 7: Results: Affinity to FcRn of <VEGF-ANG-2> bispecific antibodies**

| | VEGFang2-0015 [affinity] | VEGFang2-0016 [affinity] | VEGFang2-0012 [affinity] | VEGFang2--0201 [affinity] |
|---|---|---|---|---|
| Human FcRn | 0.8 µM | no binding | no binding | 0.8 µM |
| Cyno FcRn | 0.9 µM | no binding | no binding | 1.0 µM |
| Mouse FcRn | 0.2 µM | no binding | no binding | 0.2 µM |

**Table 8: Results Binding to FcgammaRI – IIIa**

| | VEGFang2-0015 | VEGFang2-0016 | VEGFang2-0012 | VEGFang2 -0201 |
|---|---|---|---|---|
| FcγRIa | No binding | No binding | Binding | Binding |
| FcγRIIa | No binding | No binding | No binding | Binding |
| FcγRIIIa | No binding | No binding | No binding | Binding |

**Table 9: Results: Independent binding of VEGF- and Ang2 to <VEGF-ANG-2> bispecific antibodies**

| | 1) Ang2 [RUmax] | 2) VEGF [RUmax] | 3) first VEGF then Ang2 [RUmax] | 4) first Ang2 then VEGF [RUmax] | 5) Coinjection Ang2+VEGF [RUmax] |
|---|---|---|---|---|---|
| VEGFang2-0016 | 174 | 50 | 211 | 211 | 211 |
| VEGFang2-0012 | 143 | 43 | 178 | 177 | 178 |

## Example 4

## Mass spectrometry

This section describes the characterization of <VEGF-ANG-2> bispecific antibodies with emphasis on the correct assembly. The expected primary structures were confirmed by electrospray ionization mass spectrometry (ESI-MS) of the deglycosylated, and intact or IdeS-digested (IgG-degrading enzyme of S. pyogenes) <VEGF-ANG-2> bispecific antibodies. The IdeS-digestion was performed with 100 µg purified antibody incubated with 2 µg IdeS protease (Roche) in 100 mmol/L $NaH_2PO_4$ / $Na_2HPO_4$, pH 7.1 at 37°C for 5 h. Subsequently, the antibodies were deglycosylated with N-Glycosidase F, Neuraminidase and O-glycosidase (Roche) in 100 mmol/L $NaH_2PO_4$ / $Na_2HPO_4$, pH 7.1 at 37°C for up to 16 h at a protein concentration of 1 mg/ml and subsequently desalted via HPLC on a Sephadex G25 column (GE Healthcare). The total mass was determined via ESI-MS on a maXis 4G UHR-QTOF MS system (Bruker Daltonik) equipped with a TriVersa NanoMate source (Advion).

The masses obtained for the IdeS-digested, deglycosylated (Table 10), or intact, deglycosylated (Table 11) molecules correspond to the predicted masses deduced from the amino acid sequences for the <VEGF-ANG-2> bispecific antibodies consisting of two different light chains $LC_{Ang2}$ and $LC_{Lucentis}$, and two different heavy chains $HC_{Ang2}$ and $HC_{Lucentis}$.

**Table 10: Masses of the deglycosylated and IdeS-digested bispecific <VEGF/ANG2> antibodies VEGFang2-0201 ( without AAA mutation) and VEGFang2-0012 (with AAA mutation)**

| Sample | F(ab')2 of the VEGF-ANG-2> bispecific antibody | | Deglycosylated Fc of the VEGF-ANG-2> bispecific antibody | |
|---|---|---|---|---|
| | Predicted Average Mass [Da] | Observed Average Mass [Da] | Predicted Average Mass [Da] | Observed Average Mass [Da] |
| VEGFang2-0201 | 99360.8 | 99360.7 | 47439.2 | 47430.1 |
| VEGFang2-0012 | 99360.8 | 99361.1 | 47087.7 | 47082.0 |

**Table 11: Masses of the deglycosylated <VEGF/ANG2> antibodies VEGFang2-0016 ( with AAA mutation) and VEGFang2-0015 (without AAA mutation)**

| | Deglycosylated VEGF-ANG-2> bispecific antibody | |
|---|---|---|
| | Predicted Average Mass [Da] | Observed Average Mass [Da] |
| VEGFang2-0016 | 146156.9 | 146161.2 |
| VEGFang2-0015 | 146505.3 | 146509.4 |

## Example 5

## Fc-Rn Chromatography

### Coupling to streptavidin sepharose:

One gram streptavidin sepharose (GE Healthcare) was added to the biotinylated and dialyzed receptor and incubated for two hours with shaking. The receptor derivatized sepharose was filled in a 1 ml XK column (GE Healthcare).

### Chromatography using the FcRn affinity column:

Conditions:
column dimensions: 50 mm x 5 mm

bed height: 5 cm
loading: 50 µg sample
equilibration buffer: 20 mM MES, with 150 mM NaCl, adjusted to pH 5.5
elution buffer: 20 mM Tris/HCl, with 150 mM NaCl, adjusted to pH 8.8
elution: 7.5 CV equilibration buffer, in 30 CV to 100 % elution buffer, 10 CV elution buffer

**Hu FcRn affinity column chromatography**

In the following table retention times of <VEGF-ANG-2> bispecific antibodies on affinity columns comprising human FcRn are given. Data were obtained using the conditions above. In the following Table retention times of <VEGF-ANG-2> bispecific antibodies on human FcRn are given.

**Table 12: Results: retention times of <VEGF-ANG-2> bispecific antibodies**

| antibody | retention time [min] |
|---|---|
| VEGFAng2-0015 (without AAA mutation ) | 78.5 |
| VEGFAng2-0201 (without AAA mutation ) | 78.9 |
| VEGFAng2-0012 (with AAA mutation ) | 2.7 (Void-peak) |
| VEGFAng2-0016 (with AAA mutation ) | 2.7 (Void-peak) |

## Example 6

## Pharmacokinetic( PK ) properties

### PK data with Fc-Rn mice transgenic for human FcRn

### In life phase

The study included female C57BL/6J mice (background); mouse FcRn deficient, but hemizygous transgenic for human FcRn (huFcRn, line 276 -/tg)

### Part 1

All mice were injected once intravitreally into the right eye with 2 µL/animal of the appropriate solution (i.e. 21 µg compound/animal (VEGFAng2-0015 (without

AAA mutation ) or 23.6 µg compound/animal (VEGFAng2-0016 (with AAA mutation ).

Mice were allocated to 2 groups with 6 animals each. Blood samples are taken from group 1 at 2, 24 and 96 hours and from group 2 at 7, 48 and 168 hours after dosing.

Injection into the vitreous of the right mouse eye was performed by using the NanoFil Microsyringe system for nanoliter injection from World Precision Instruments, Inc., Berlin, Germany. Mice were anesthetized with 2.5% Isoflurane and for visualization of the mouse eye a Leica MZFL 3 microscope with a 40 fold magnification and a ring-light with a Leica KL 2500 LCD lightning was used. Subsequently, 2 µL of the compound were injected using a 35-gauge needle.

Blood was collected via the retrobulbar venous plexus of the **contralateral eye** from each animal for the determination of the compound levels in serum.

Serum samples of at least 50 µl were obtained from blood after 1 hour at RT by centrifugation (9300xg) at 4°C for 3 min. Serum samples were frozen directly after centrifugation and stored frozen at–80°C until analysis. Treated eyes of the animals of group 1 were isolated 96 hours after treatment and of the animals of group 2 168 hours after treatment. Samples were stored frozen at –80°C until analysis.

**Part 2**

All mice were injected once intravenously via the tail vein with 200 µL/animal of the appropriate solution (i.e. 21 µg compound/animal (VEGFAng2-0015 (without AAA mutation) or 23.6 µg compound/animal (VEGFAng2-0016 (with AAA mutation).

Mice were allocated to 2 groups with 5 animals each. Blood samples are taken from group 1 at 1, 24 and 96 hours and from group 2 at 7, 48 and 168 hours after dosing. Blood was collected via the retrobulbar venous plexus from each animal for the determination of the compound levels in serum.

Serum samples of at least 50 µl were obtained from blood after 1 hour at RT by centrifugation (9300xg) at 4°C for 3 min. Serum samples were frozen directly after centrifugation and stored frozen at –80°C until analysis.

### Preparation of whole eye lysates (mice)

The eye lysates were gained by physico-chemical disintegration of the whole eye from laboratory animals. For mechanical disruption, each eye was transferred into a 1.5-mL micro vial with conical bottom. After freeze and thawing, the eyes were washed with 1mL cell washing buffer once (Bio-Rad, Bio-Plex Cell Lysis Kit, Cat. No. 171-304011). In the following step, 500µL of freshly prepared cell lysis buffer were added and the eyes were grinded using a 1.5mL tissue grinding pestle (Kimble Chase, 1.5mL pestle, Art. No. 749521-1500). The mixture was then frozen and thawed five times and grinded again. To separate lysate from remaining tissue the samples were centrifuged for 4 min at 4500 g. After centrifuging the supernatant was collected and stored at -20°C until further analysis in the quantification ELISA.

### Analysis

The concentrations of the <VEGF/ANG2> antibodies in mice serum and eye lysates were determined with an enzyme linked immunosorbent assay (ELISA)

For quantification of <VEGF/ANG2> antibodies in mouse serum samples and eye lysates, a standard solid-phase serial sandwich immunoassay with biotinylated and digoxigenated monoclonal antibodies used as capture and detection antibodies was performed. To verify the integrity of the bispecifity of the analyte the biotinylated capture antibody recognizes the anti-VEGF-binding site whereas the digoxigenated detection antibody will bind to the anti-Ang2 binding site of the analyte. The bound immune complex of capture antibody, analyte and detection antibody on the solid phase of the streptavidin coated micro titer plate (SA-MTP) is then detected with a horseradish-peroxidase coupled to an anti-digoxigenin antibody. After washing unbound material from the SA-MTP and addition of ABTS-substrate, the gained signal is proportional to the amount of analyte bound on the solid phase of the SA-MTP. Quantification is then done by converting the measured signals of the samples into concentrations referring to calibrators analyzed in parallel.

In a first step the SA-MTP was coated with 100µL/well of biotinylated capture antibody solution (mAb<Id<VEGF>>M-2.45.51-IgG-Bi(DDS)) with a concentration of 1µg/mL for one hour at 500 rpm on a MTP-shaker. Meanwhile calibrators, QC-samples and samples were prepared. Calibrators and QC-samples are diluted to 2% serum matrix; samples were diluted until the signals were within the linear range of the calibrators.

After coating the SA-MTP with capture antibody, the plate was washed three times with washing buffer and 300µL/well. Subsequently 100µl/well of the calibrators, QC-samples and samples were pipetted on the SA-MTP and incubated again for one hour at 500 rpm. The analyte was now bound with its anti-VEGF binding site via the capture antibody to the solid phase of the SA-MTP. After incubation and removal of unbound analyte by washing the plate 100µL/well of the first detection antibody (mAb<Id-<Ang2>>M-2.6.81-IgG-Dig(XOSu)) with a concentration of 250ng/mL was added to the SA-MTP. Again, the plate was incubated for one hour at 500 rpm on a shaker. After washing, 100µL/well of the second detection antibody (pAb<Digoxigenin>S-Fab-POD (poly)) at a concentration of 50 mU/mL was added to the wells of the SA-MTP and the plate was incubated again for one hour at 500 rpm. After a final washing step to remove excess of detection antibody, 100 µL/well substrate (ABTS) is added. The antibody-enzyme conjugate catalyzes the color reaction of the ABTS® substrate. The signal was then measured by an ELISA reader at 405 nm wavelength (reference wavelength: 490 nm ([405/490] nm)).

## Pharmacokinetic Evaluation

The pharmacokinetic parameters were calculated by non-compartmental analysis, using the pharmacokinetic evaluation program WinNonlinTM (Pharsight), version 5.2.1.

## Results: A) Serum concentrations

Results for serum concentrations are shown in Tables 13 to 16 and Fig. 7B to 7C

**Table 13: VEGFAng2-0015 (without AAA mutation ): Comparison of serum concentrations after intravitreal and intravenous application**

| | Serum concentration after intravitreal application | Serum concentration after intravenous application |
|---|---|---|
| ID | Average conc. [µg/mL] | Average conc. [µg/mL] |
| 1h | | 17.7 |
| 2h | 9.8 | |
| 7h | 10.4 | 12.1 |
| 24h | 6.4 | 8.3 |
| 48h | 6.5 | 6.9 |
| 96h | 3.4 | 4.1 |
| 168h | 2.9 | 2.7 |

**Table 14: VEGFAng2-0016 (with AAA mutation): Comparison of serum concentrations after intravitreal and intravenous application**

| | Serum concentration after intravitreal application | Serum concentration after intravenous application |
|---|---|---|
| ID | Average conc. [µg/mL] | Average conc. [µg/mL] |
| 1h | | 18.4 |
| 2h | 7.0 | |
| 7h | 8.7 | 10.0 |
| 24h | 2.2 | 3.3 |
| 48h | 1.0 | 1.0 |
| 96h | 0.1 | 0.1 |
| 168h | 0.0 | 0.0 |

**Table 15: VEGFang2-0015 ( without AAA mutation) and VEGFang2-0016 ( with AAA mutation) : Comparison of <u>serum concentrations</u> after <u>intravitreal application</u>)**

| | **VEGFang2-0015 ( without AAA mutation)** | **VEGFang2-0016 ( with AAA mutation)** |
|---|---|---|
| ID | **Average conc. [µg/mL]** | **Average conc. [µg/mL]** |
| 2h | **9.8** | **7.0** |
| 7h | **10.4** | **8.7** |
| 24h | **6.4** | **2.2** |
| 48h | **6.5** | **1.0** |
| 96h | **3.4** | **0.1** |
| 168h | **2.9** | **0.0** |

**Table 16: VEGFang2-0015 (without AAA mutation) and VEGFang2-0016 (with AAA mutation) : Comparison of serum concentrations after intravenous application**

| | **VEGFang2-0015 ( without AAA mutation)** | **VEGFang2-0016 ( with AAA mutation)** |
|---|---|---|
| ID | **Average conc. [µg/mL]** | **Average conc. [µg/mL]** |
| 1h | **17.7** | **18.4** |
| 7h | **12.1** | **10.0** |
| 24h | **8.3** | **3.3** |
| 48h | **6.9** | **1.0** |
| 96h | **4.1** | **0.1** |
| 168h | **2.7** | **0.0** |

**Results: B) Concentrations in eye-lysates of left and right eyes**

Results for concentrations in eye lysates are shown in Tables 17 to 18 and Figures 7D to 7E

**Table 17a: Concentrations of VEGFang2-0015 (without AAA mutation) in eye lysates after intra vitreal application into right eye**

| Mean conc. values from n=6 mice | | |
|---|---|---|
| ID | | mean conc. [ng/mL] |
| 96h | Left eye | 8.7 |
| | Right eye | 46.1 |
| 168h | Left eye | 4.3 |
| | Right eye t | 12.9 |

**Table 17b: Concentrations of VEGFang2-0015 (without AAA mutation) in eye lysates after intravenous application**

| Mean conc. values from n=5 mice | | |
|---|---|---|
| ID | | mean conc. [ng/mL] |
| 96h | Left eye | 4.2 |
| | Right eye | 7.5 |
| 168h | Left eye | 3.4 |
| | Right eye | 6.1 |

**Table 18a: Concentrations of VEGFang2-0016 ( with AAA mutation) in eye lysates after intra vitreal application into right eye**

| Mean conc. values from n=5 mice | | |
|---|---|---|
| ID | | mean conc. [ng/mL] |
| 96h | Left eye | 0.3 |
| | Right eye | 34.5 |
| 168h | Left eye | 0.1 |
| | Right eye | 9.0 |

**Table 18b: Concentrations of VEGFang2-0016 ( with AAA mutation) in eye lysates after intravenous application**

| Mean conc. values from n=5 mice | | |
|---|---|---|
| ID | | mean conc. [ng/mL] |
| 96h | Left eye | 0.0 |
| | Right eye | 0.1 |
| 168h | Left eye | 0.0 |
| | Right eye | 0.1 |

**Summary of Results:**

After intravitreal application the bispecific <VEGF/ANG2> antibody according to the invention VEGFang2-0016 ( with AAA mutation) shows similar concentrations (after 96 and 168 hours) in the eye lysates as compared to the bispecific <VEGF/ANG2> antibody without AAA mutation VEGFang2-0015.

Also after intravitreal application the bispecific <VEGF/ANG2> antibody according to the invention VEGFang2-0016 (with AAA mutation) shows in addition a faster clearance and shorter half-life in the serum as compared to the bispecific <VEGF/ANG2> antibody without AAA mutation VEGFang2-0015.

## Example 7

### Mouse cornea micropocket angiogenesis assay

To test the anti-angiogenic effect bispecific <VEGF/ANG2> antibody with the respective anti-VEGF VH and VL of SEQ ID NO: 7 and 8 and the anti-ANG2 VH and VL of SEQ ID NO: 15 and 16 on VEGF-induced angiogenesis in vivo, we perform the mouse corneal angiogenesis assay. In this assay a VEGF soaked Nylaflo disc is implanted into a pocket of the avascular cornea at a fixed distance to the limbal vessels. Vessels immediately grow into the cornea towards the developing VEGF gradient. 8 to 10 weeks old female Balb/c mice were purchased from Charles River, Sulzfeld, Germany. The protocol is modified according to the method described by Rogers, M.S., et al., Nat. Protoc. 2 (2007) 2545-2550. Briefly, micropockets with a width of about 500 µm are prepared under a microscope at approximately 1 mm from the limbus to the top of the cornea using a surgical blade and sharp tweezers in the anesthetized mouse. The disc (Nylaflo®, Pall Corporation, Michigan) with a diameter of 0.6 mm is implanted and the surface of the implantation area was smoothened. Discs are incubated in corresponding growth factor or in vehicle for at least 30 min. After 3, 5 and 7 days (or alternatively only after 3, 5 or 7 days) , eyes are photographed and vascular response is measured. The assay is quantified by calculating the percentage of the area of new vessels per total area of the cornea.

The discs are loaded with 300 ng VEGF or with PBS as a control and implanted for 7 days. The outgrowth of vessels from the limbus to the disc is monitored over time on day 3, 5 and/or 7. One day prior to disc implantation the antibodies are administered intravenously at a dose of 10 mg/kg (due to the intravenous application the serum-stable VEGFang2-0015 (without AAA mutation) which only differs from VEGFang2-0016 by the AAA mutation and has the same anti-VEGF and anti-ANG2 VHs and VLs to mediate efficacy, is used as surrogate) for testing the anti-angiogenic effect on VEGF-induced angiogenesis in vivo. Animals in the control group receive vehicle. The application volume is 10 ml/kg.

## CLAIMS

1. 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 subclass comprising the mutations I253A, H310A, and H435A, numbering according to EU Index of Kabat.

2. The bispecific antibody according to claim 1, 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.

3. The bispecific antibody according to claim 1, wherein the constant heavy chain region of IgG1 subclass further comprises the mutations L234A , L235A and P329G, numbering according to EU Index of Kabat.

4. The bispecific antibody according to any one of 1 to 3 for use in the treatment of an ocular vascular disease.

5. The bispecific antibody according to claim 4, wherein the antibody is administered via intravitreal application.

6. A nucleic acid encoding a bispecific antibody according to any one of claims 1 to 3.

7. Expression vector containing said nucleic acid according claim 6 capable of expressing said nucleic acid in a prokaryotic or eukaryotic host cell.

8. A prokaryotic or eukaryotic host cell comprising a vector according to claim 7.

9. A method for the preparation of a bispecific antibody according to any one of claims 1 to 3

   comprising the steps of

   a) transforming a host cell with a vector comprising a nucleic acid molecule 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.

10. A bispecific antibody obtained by the method of claim 9.

11. 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.

12. 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.

13. The bispecific, bivalent antibody according to any one of claims 11 to 12 for use in the treatment of an ocular vascular disease.

14. The bispecific, bivalent antibody according to claim 13, wherein the antibody is administered via intravitreal application.

15. A pharmaceutical composition for treating an ocular vascular disease comprising a bispecific antibody according to any one of claims 1 to 3, or a bispecific, bivalent antibody according to any one of claims 11 to 12.

16. The pharmaceutical composition of claim 15, wherein the pharmaceutical composition is administered via intravitreal application.

17. Use of the bispecific antibody according to any one of claims 1 to 3 and 11 to 12, for the manufacture of a medicament for the treatment of an ocular vascular disease.

18. The use of claim 17 wherein the antibody is administered via intravitreal application.

19. A method of treatment of a patient suffering from an ocular vascular disease by administering a therapeutically effective amount of an antibody according to any one of claims 1 to 3 and 11 to 12 to a patient in need of such treatment.

20. The method of claim 19, wherein the antibody is administered via intravitreal application.

**Roche Glycart AG**

**Patent Attorneys for the Applicant/Nominated Person**

**SPRUSON & FERGUSON**

Fig. 1

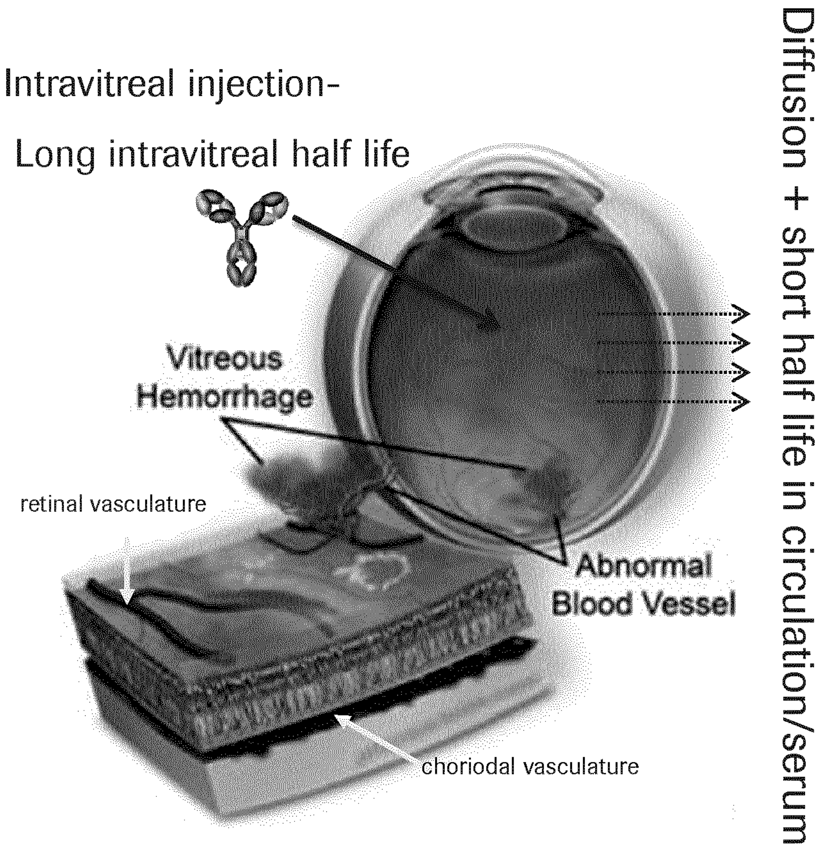

Intravitreal injection-
Long intravitreal half life
Vitreous Hemorrhage
retinal vasculature
Abnormal Blood Vessel
choriodal vasculature
Diffusion + short half life in circulation/serum

Fig. 2

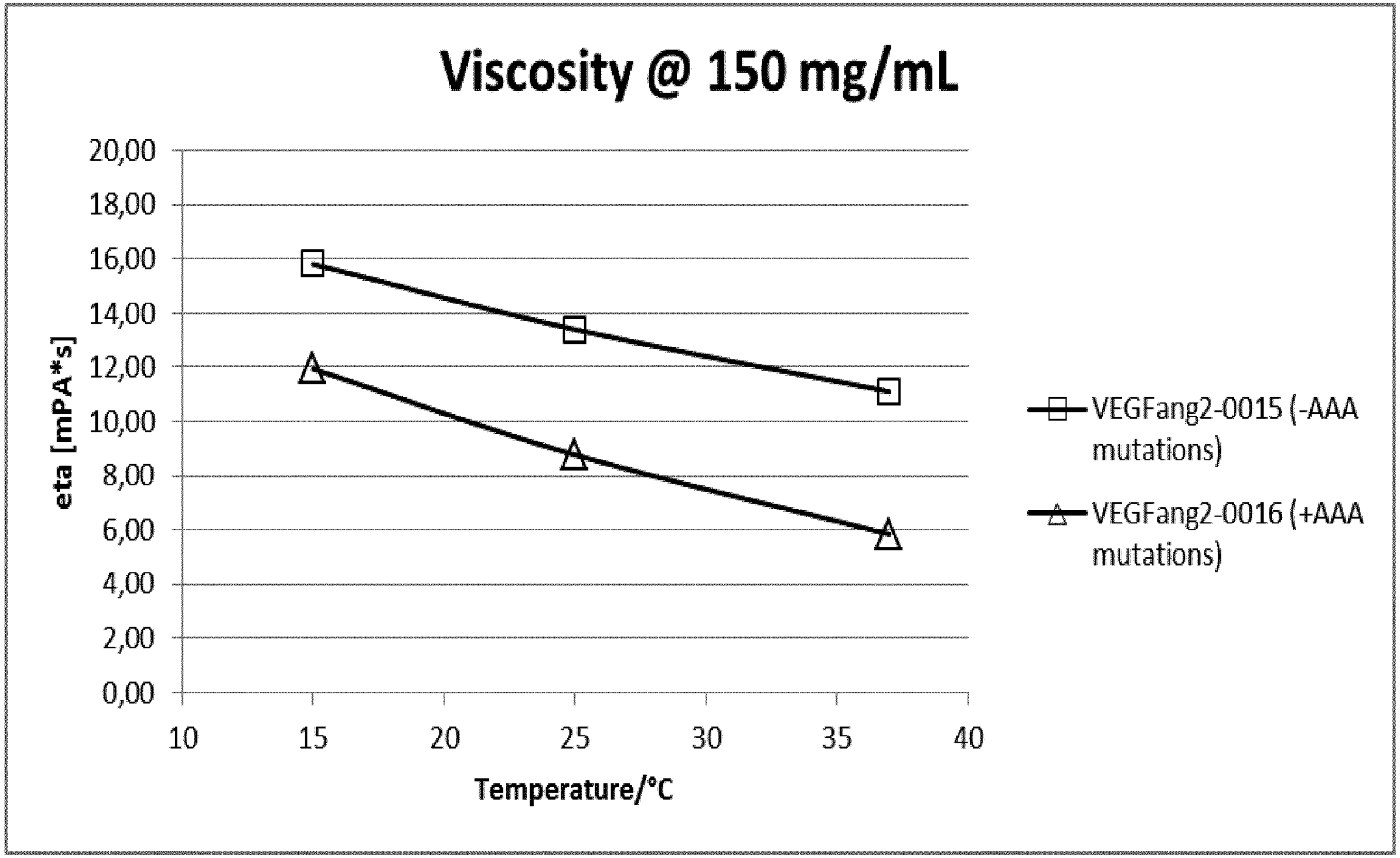

Viscosity @ 150 mg/mL
eta [mPA*s]
20,00
18,00
16,00
14,00
12,00
10,00
8,00
6,00
4,00
2,00
0,00
10
15
20
25
30
35
40
Temperature/°C
VEGFang2-0015 (-AAA mutations)
VEGFang2-0016 (+AAA mutations)

Fig. 3

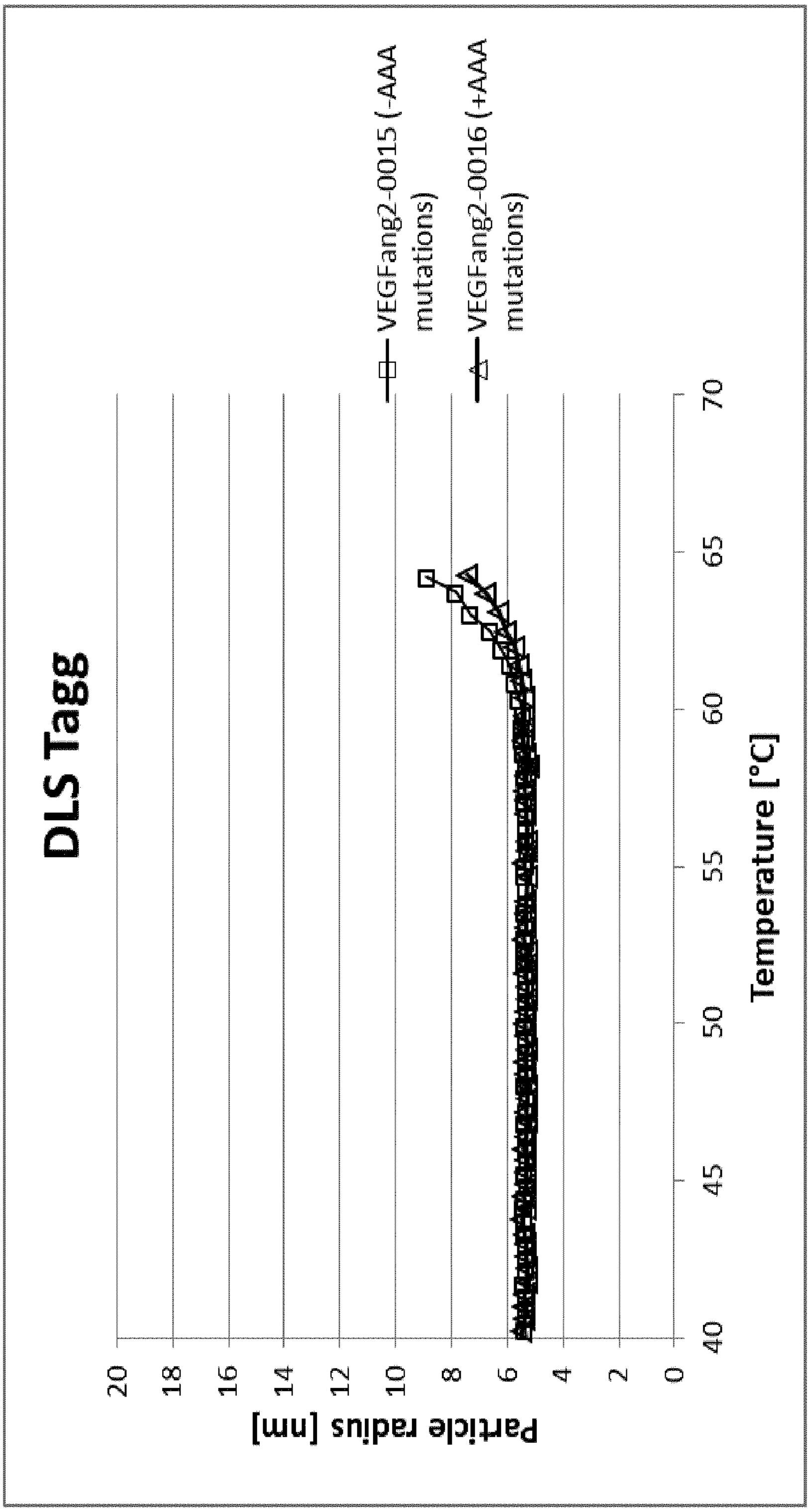

DLS Tagg
Particle radius [nm]
0
2
4
6
8
10
12
14
16
18
20
Temperature [°C]
40
45
50
55
60
65
70
VEGFang2-0015 (-AAA mutations)
VEGFang2-0016 (+AAA mutations)

Fig. 4

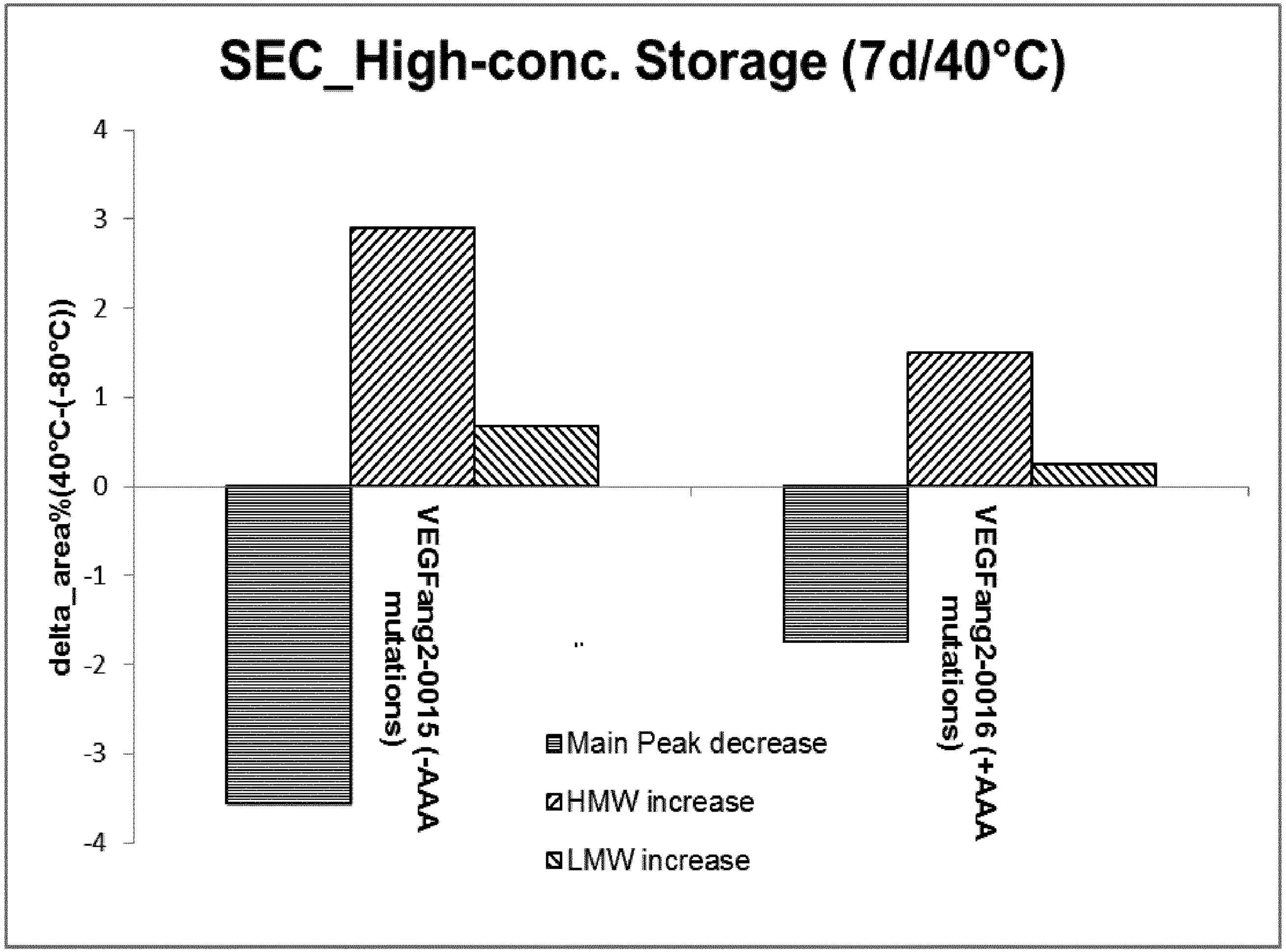

SEC_High-conc. Storage (7d/40°C)
delta_area%(40°C-(-80°C))
4
3
2
1
0
-1
-2
-3
-4
VEGFang2-0015 (-AAA mutations)
VEGFang2-0016 (+AAA mutations)
▤Main Peak decrease
▨HMW increase
▧LMW increase

Fig. 5A

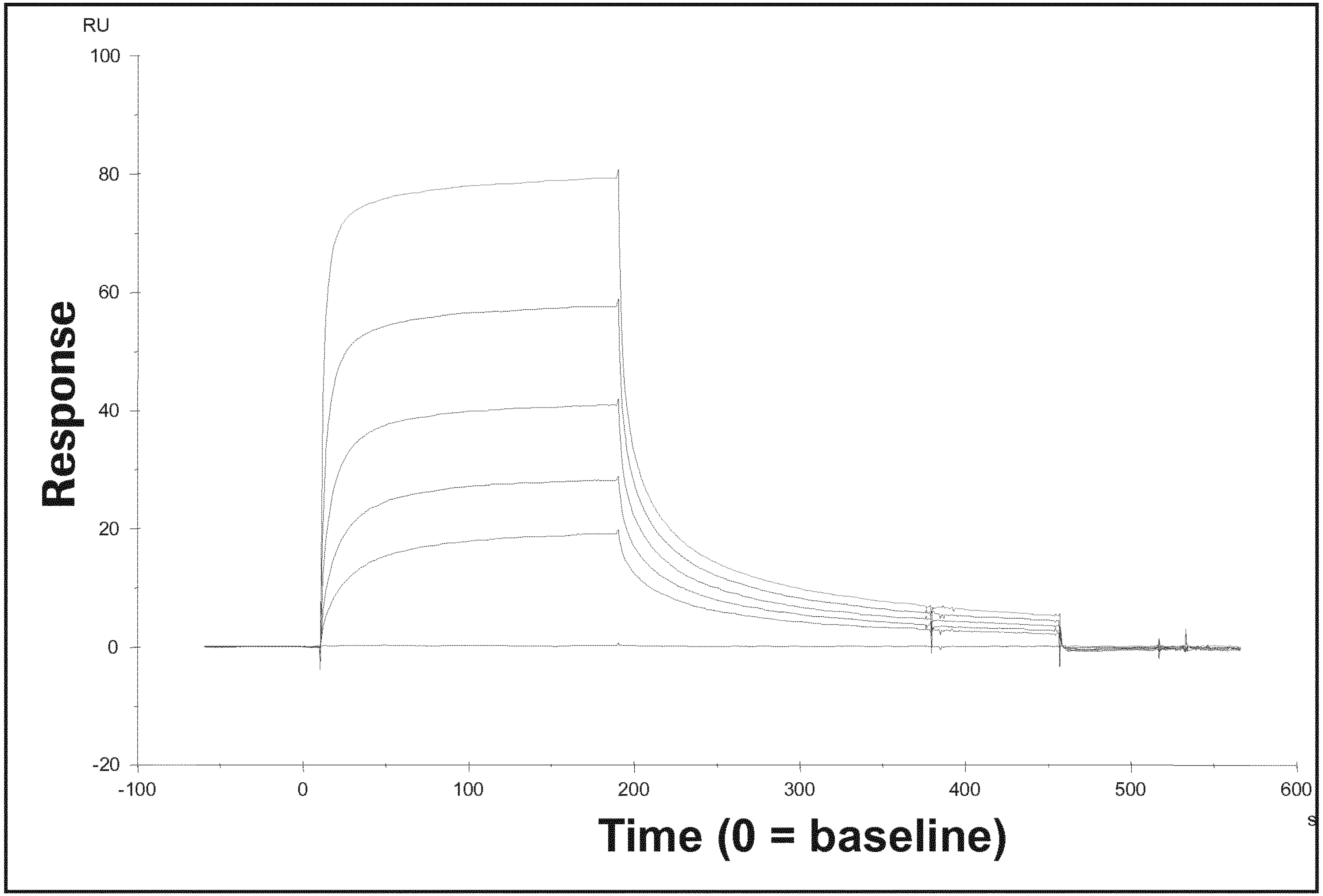

RU
100
80
60
40
20
0
-20
Response
-100
0
100
200
300
400
500
600
s
Time (0 = baseline)

Fig. 5B

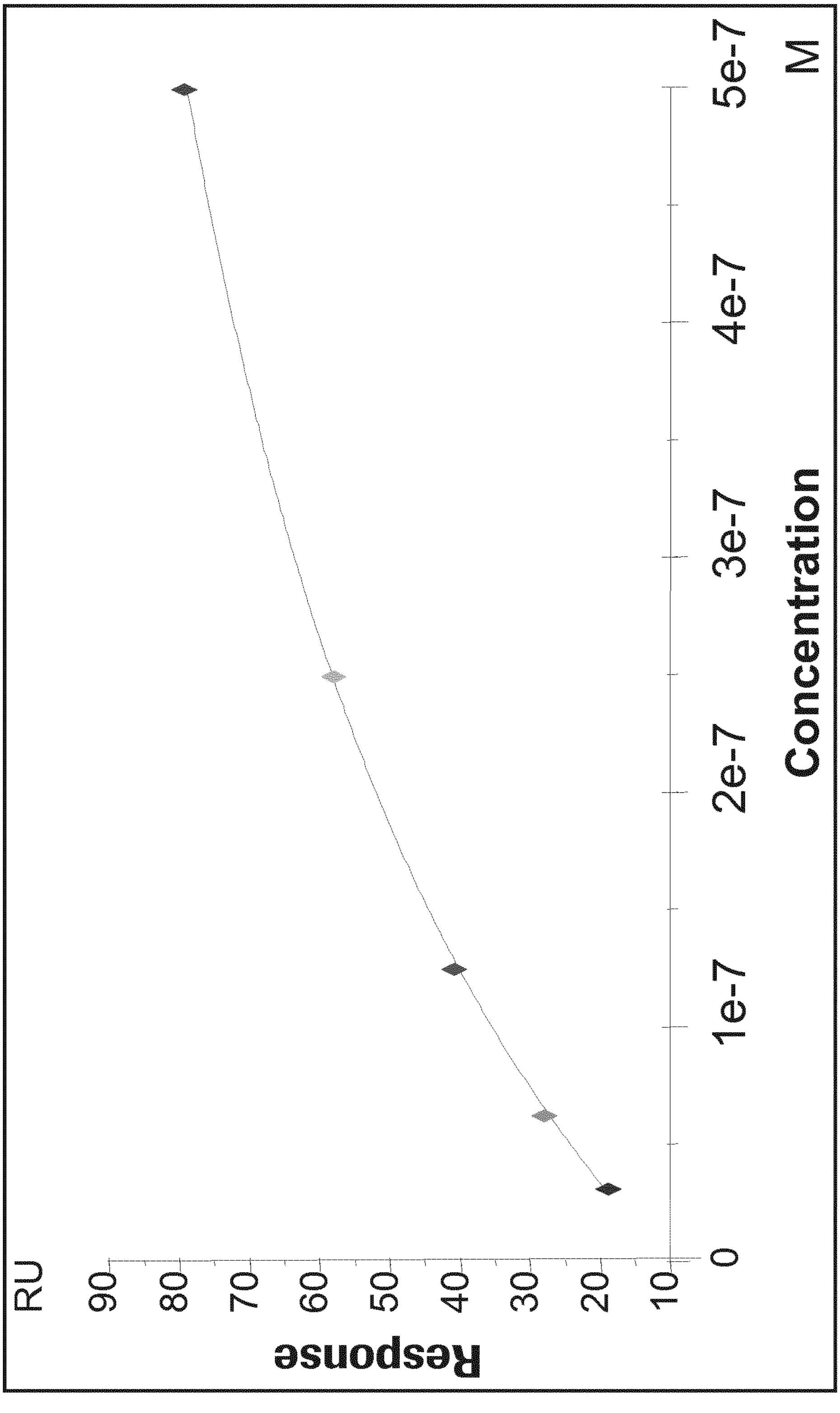

RU
Response
90
80
70
60
50
40
30
20
10
0
1e-7
2e-7
3e-7
4e-7
5e-7
M
Concentration

Fig. 5C

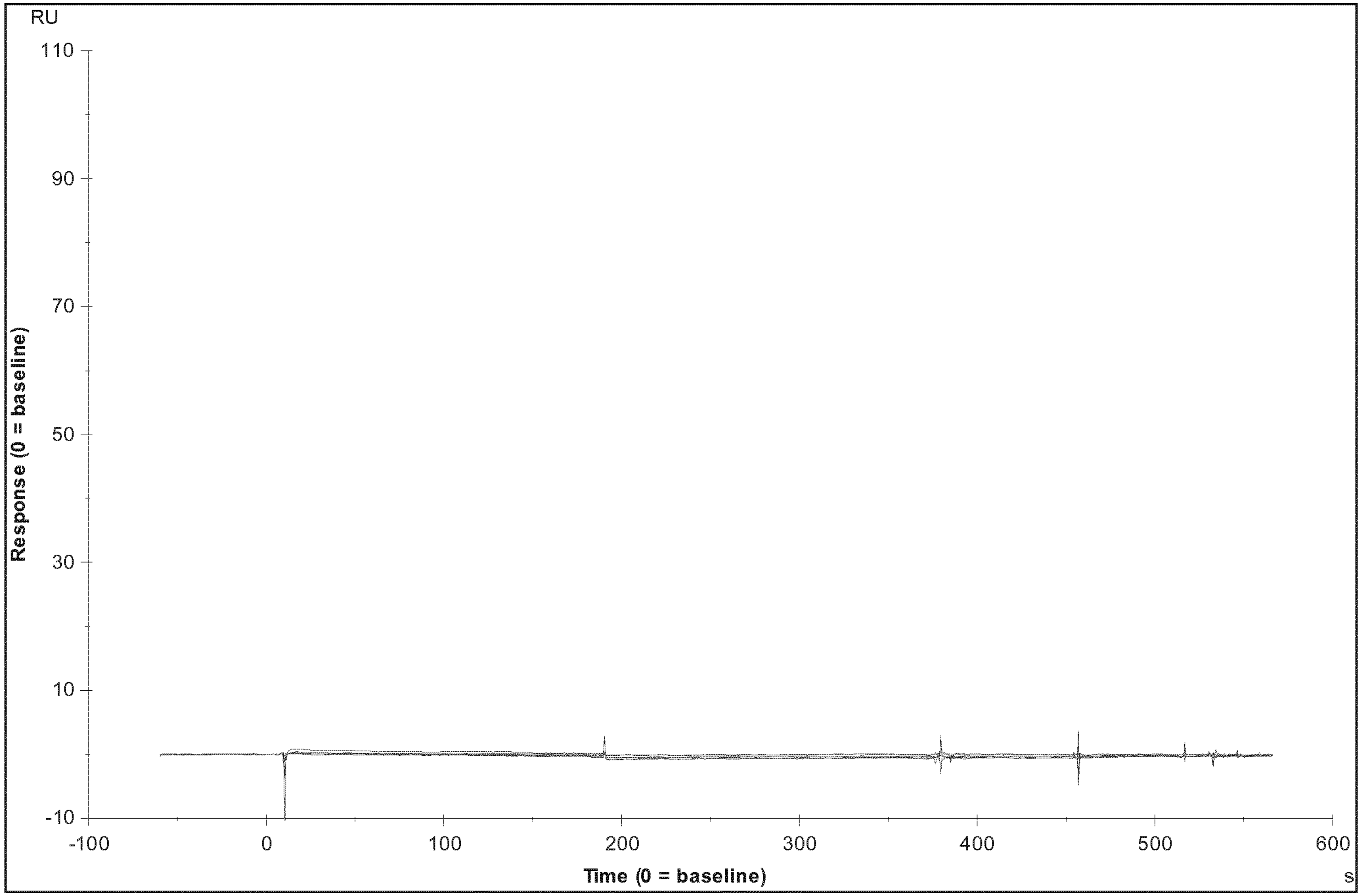

RU
110
90
70
50
30
10
-10
Response (0 = baseline)
-100
0
100
200
300
400
500
600
Time (0 = baseline)
s

Fig. 5D

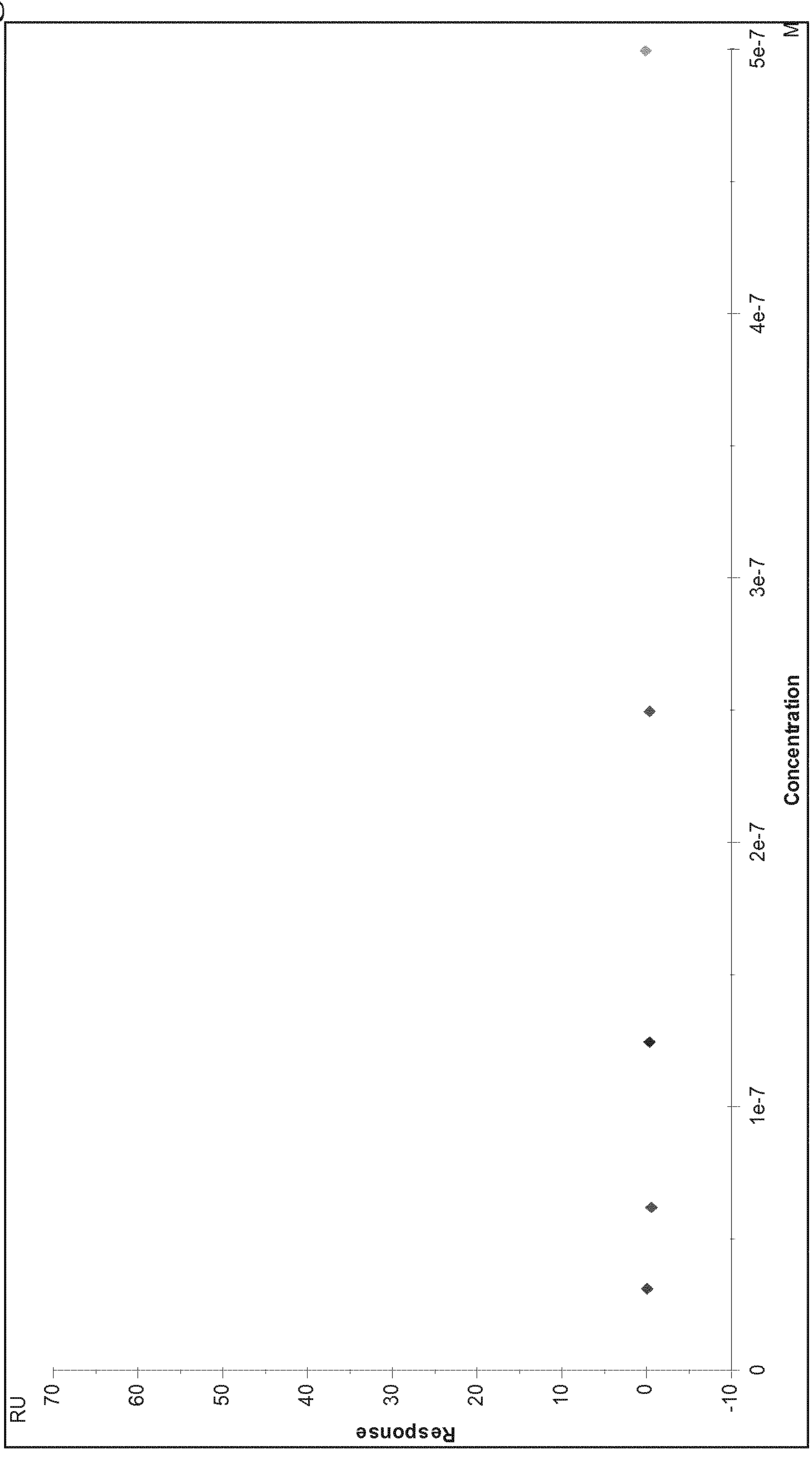

RU
Response
70
60
50
40
30
20
10
0
-10
0
1e-7
2e-7
3e-7
4e-7
5e-7
M
Concentration

Fig. 5E

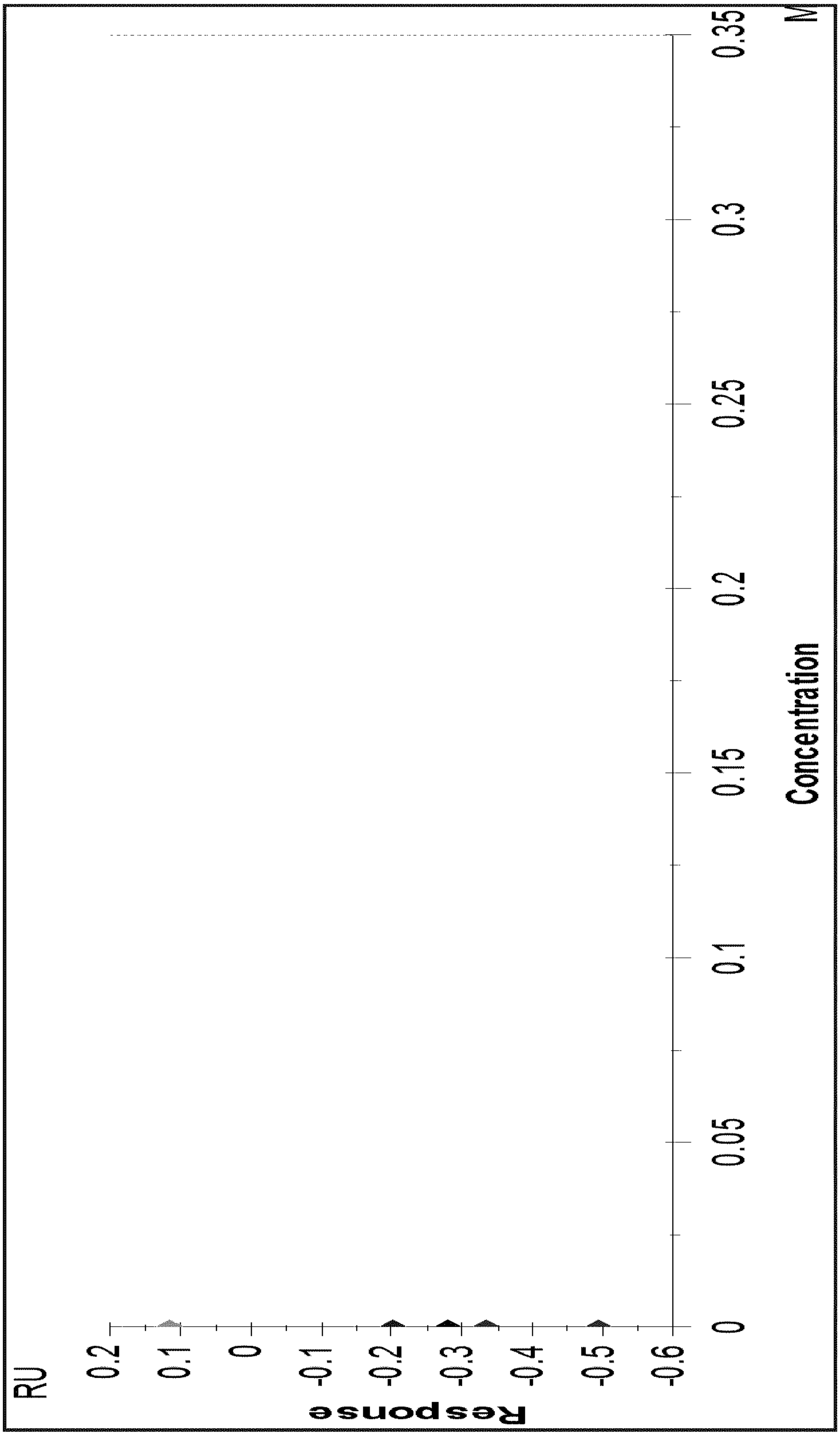

RU
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
Response
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
M
Concentration

**Fig. 6**

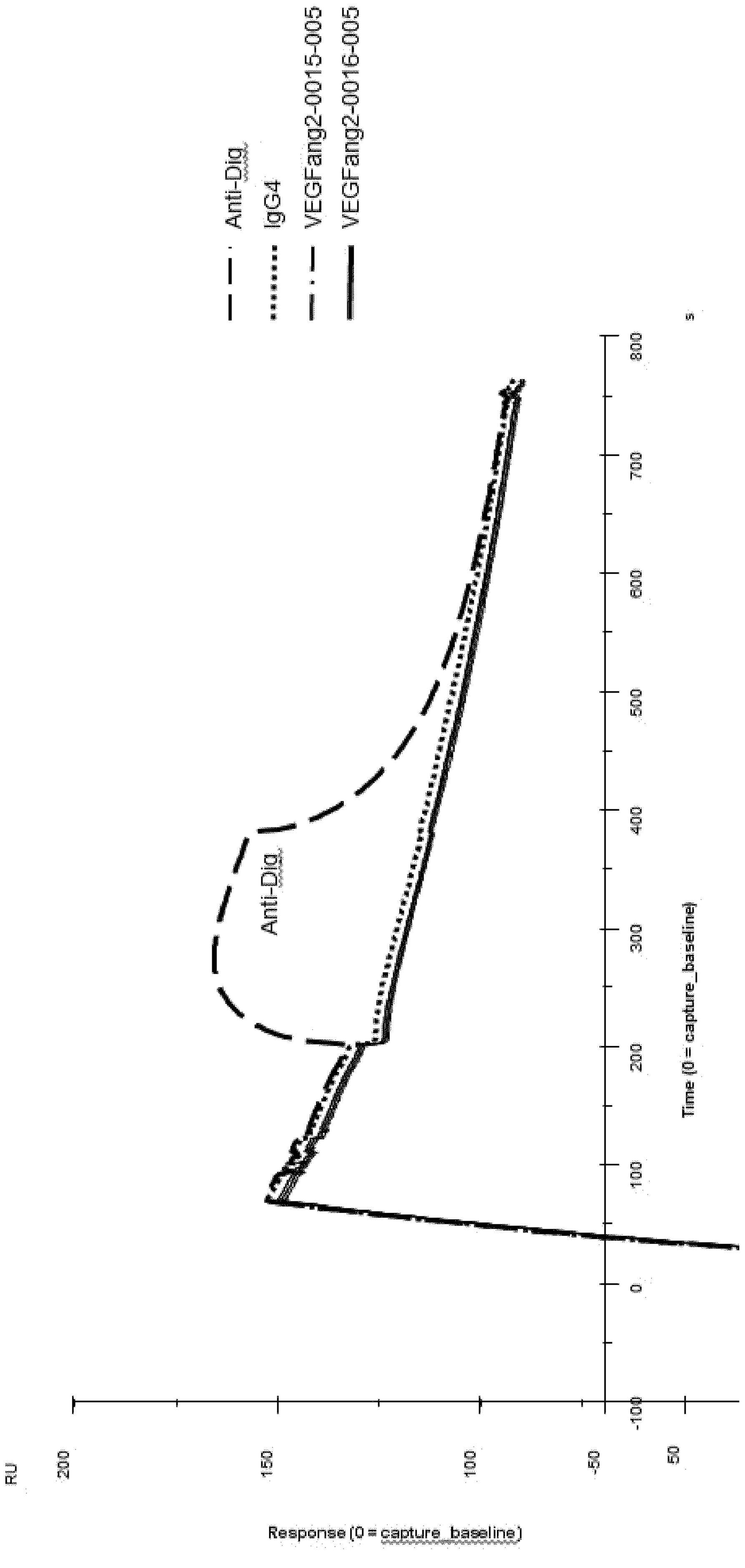

Anti-Dig
IgG4
VEGFang2-0015-005
VEGFang2-0016-005
Anti-Dig
RU
200
150
100
-50
50
Response (0 = capture_baseline)
-100
0
100
200
300
400
500
600
700
800
s
Time (0 = capture_baseline)

Fig. 7A

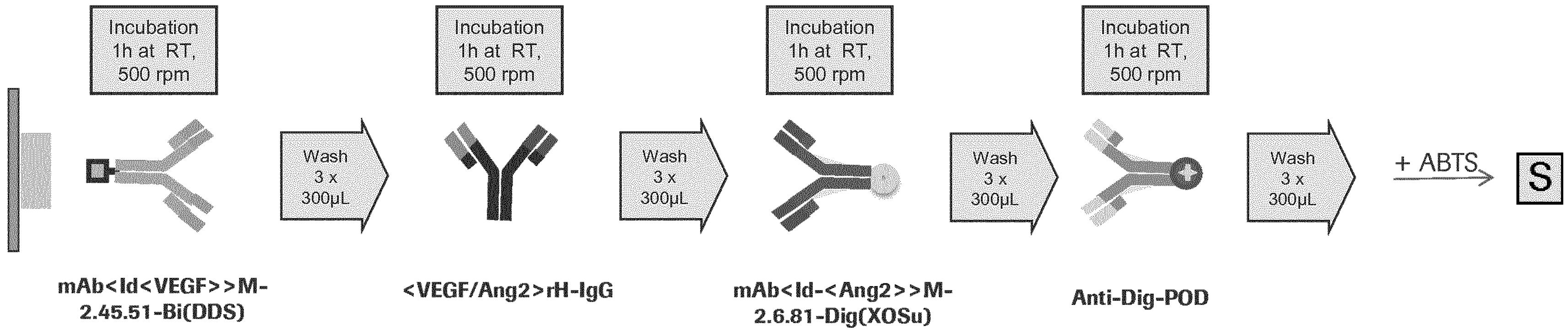

Incubation
1h at RT,
500 rpm
mAb<Id<VEGF>>M-2.45.51-Bi(DDS)
Wash
3 x
300µL
Incubation
1h at RT,
500 rpm
<VEGF/Ang2>rH-IgG
Wash
3 x
300µL
Incubation
1h at RT,
500 rpm
mAb<Id-<Ang2>>M-2.6.81-Dig(XOSu)
Wash
3 x
300µL
Incubation
1h at RT,
500 rpm
Anti-Dig-POD
Wash
3 x
300µL
+ ABTS
S

Fig. 7B

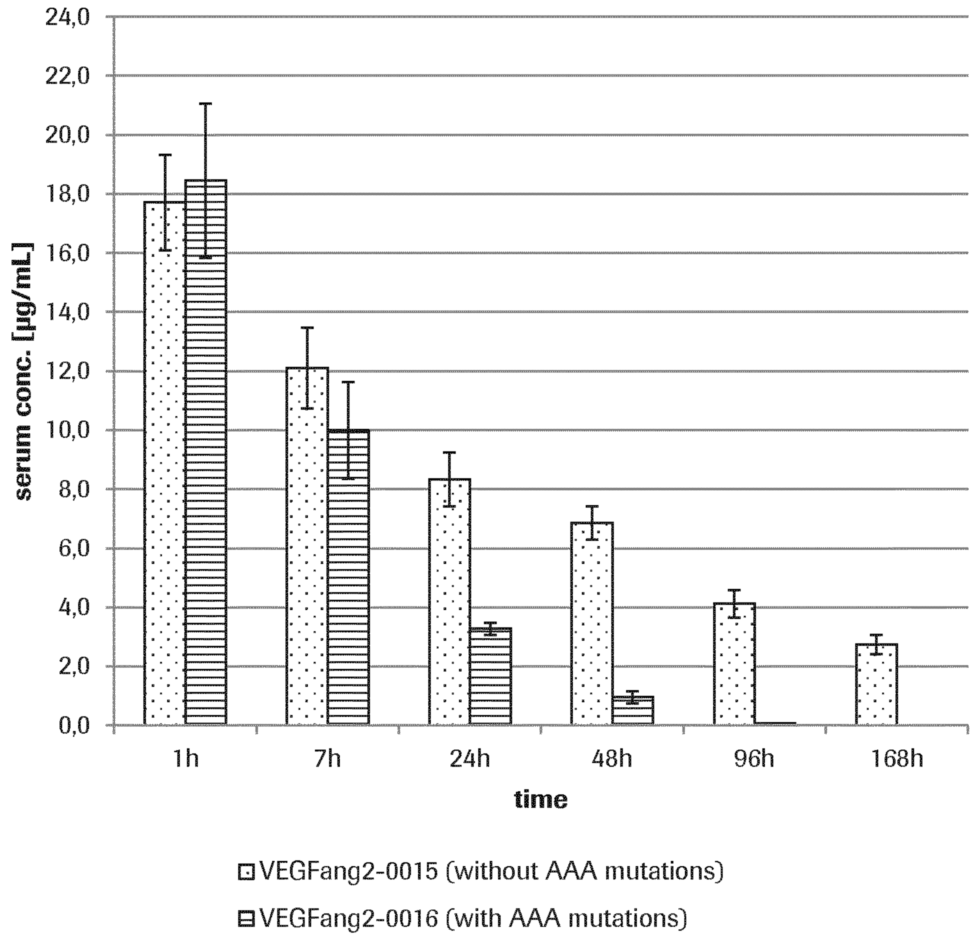

24,0
22,0
20,0
18,0
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
serum conc. [µg/mL]
1h
7h
24h
48h
96h
168h
time
⊡VEGFang2-0015 (without AAA mutations)
⊟VEGFang2-0016 (with AAA mutations)

Fig. 7C

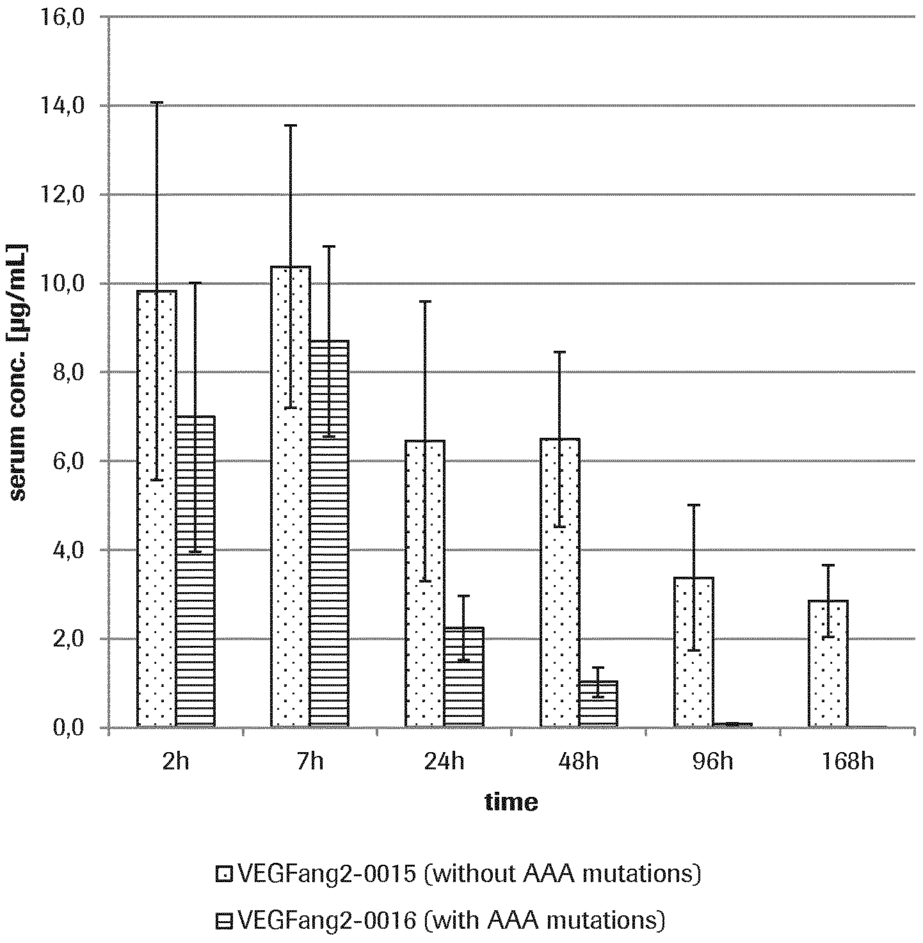

16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
serum conc. [µg/mL]
2h
7h
24h
48h
96h
168h
time
⊡VEGFang2-0015 (without AAA mutations)
⊟VEGFang2-0016 (with AAA mutations)

Fig. 7D

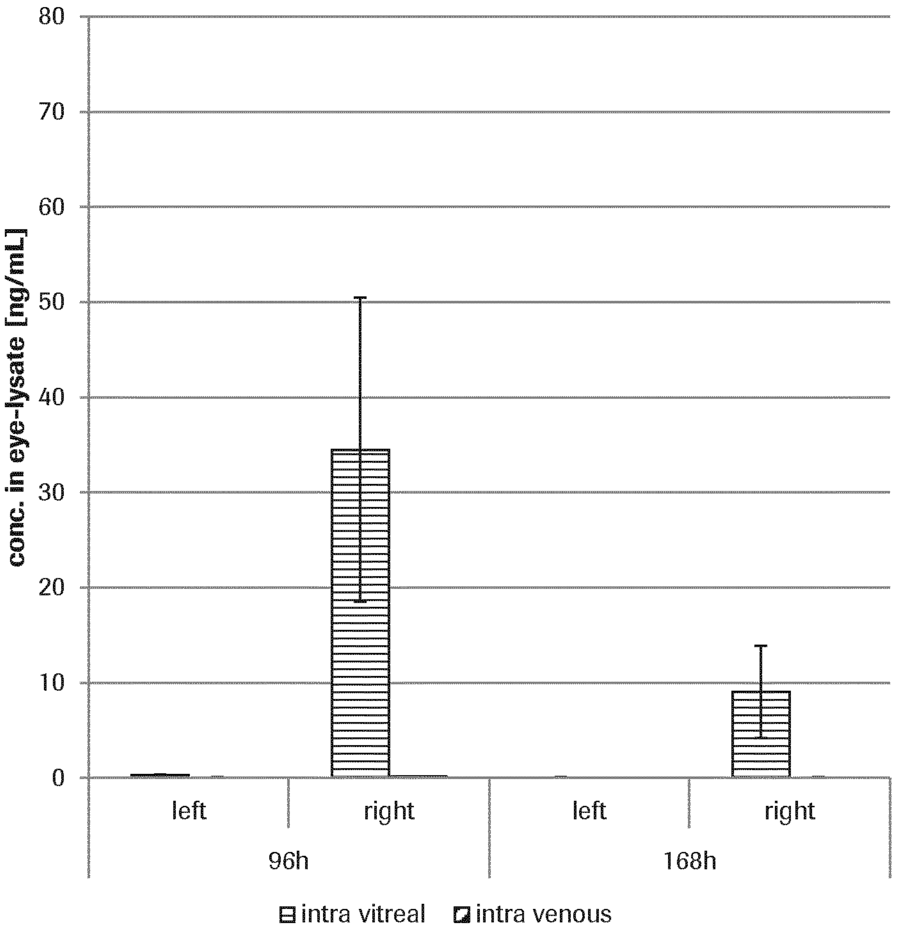
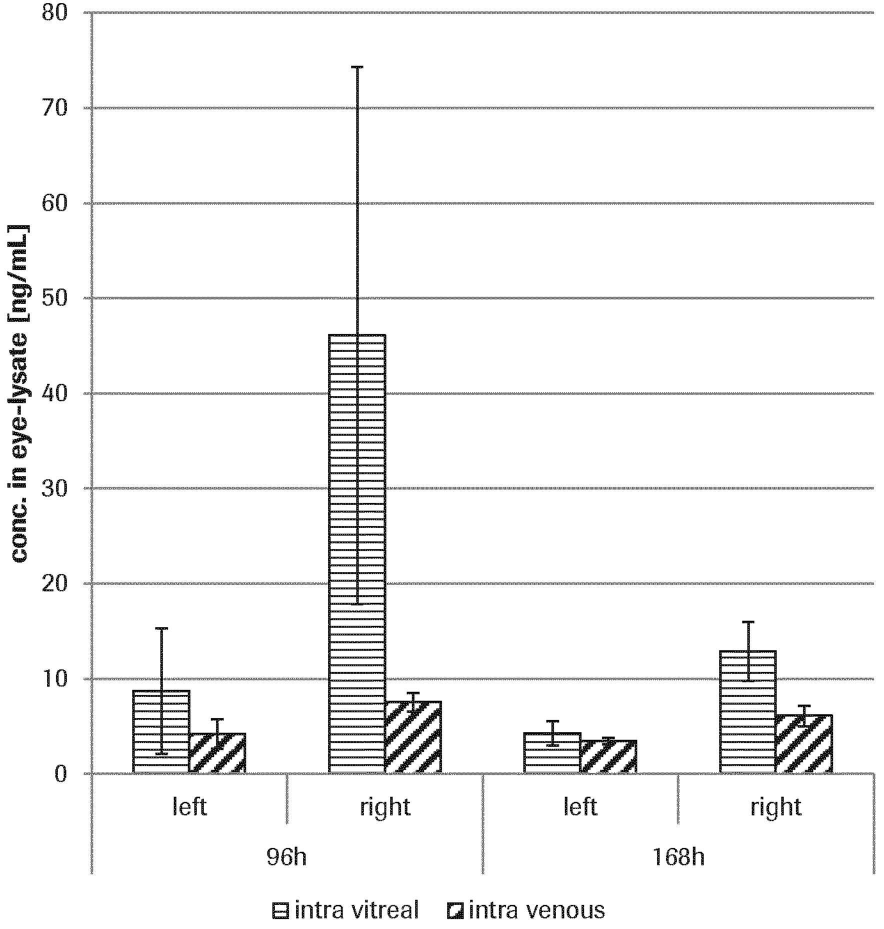

conc. in eye-lysate [ng/mL]
80
70
60
50
40
30
20
10
0
left
right
left
right
96h
168h
⊟ intra vitreal
◪ intra venous

Fig. 7E


80
70
60
50
40
30
20
10
0
conc. in eye-lysate [ng/mL]
left
right
left
right
96h
168h
⊟ intra vitreal
▨ intra venous

# SEQUENCE LISTING

```
<110>  F. Hoffmann-La Roche AG

<120>  Bispecific anti-VEGF/anti-ANG-2 antibodies and their use in the
       treatment of ocular vascular diseases

<130>  31094 WO

<150>  EP12176299.1
<151>  2012-07-13

<160>  50

<170>  PatentIn version 3.5

<210>  1
<211>  14
<212>  PRT
<213>  Artificial

<220>
<223>  heavy chain CDR3H, <VEGF>ranibizumab

<400>  1

Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
1               5                   10


<210>  2
<211>  17
<212>  PRT
<213>  Artificial

<220>
<223>  heavy chain CDR2H, <VEGF>ranibizumab

<400>  2

Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe Lys
1               5                   10                  15


Arg



<210>  3
<211>  5
<212>  PRT
<213>  Artificial

<220>
<223>  heavy chain CDR1H, <VEGF>ranibizumab

<400>  3

His Tyr Gly Met Asn
1               5


<210>  4
<211>  9
<212>  PRT
<213>  Artificial

<220>
```

```
<223>  light chain CDR3L,  <VEGF>ranibizumab

<400>  4

Gln Gln Tyr Ser Thr Val Pro Trp Thr
1               5


<210>  5
<211>  7
<212>  PRT
<213>  Artificial

<220>
<223>  light chain CDR2L,  <VEGF>ranibizumab

<400>  5

Phe Thr Ser Ser Leu His Ser
1               5


<210>  6
<211>  11
<212>  PRT
<213>  Artificial

<220>
<223>  light chain CDR1L,  <VEGF>ranibizumab

<400>  6

Ser Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn
1               5                   10


<210>  7
<211>  123
<212>  PRT
<213>  Artificial

<220>
<223>  heavy chain variable domain VH,  <VEGF>ranibizumab

<400>  7

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr
            20                  25                  30


Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60


Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
```

```
Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
            100                 105                 110


Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120


<210>  8
<211>  107
<212>  PRT
<213>  Artificial

<220>
<223>  light chain variable domain VL,  <VEGF>ranibizumab

<400>  8

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30


Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45


Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105


<210>  9
<211>  20
<212>  PRT
<213>  Artificial

<220>
<223>  heavy chain CDR3H, <ANG-2> Ang2i_LC10 variant

<400>  9

Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr Pro Gly
1               5                   10                  15


Ala Phe Asp Ile
            20


<210>  10
<211>  17
```

```
<212>  PRT
<213>  Artificial

<220>
<223>  heavy chain CDR2H, <ANG-2> Ang2i_LC10 variant

<400>  10

Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe Gln
1               5                   10                  15


Gly



<210>  11
<211>  5
<212>  PRT
<213>  Artificial

<220>
<223>  heavy chain CDR1H, <ANG-2> Ang2i_LC10 variant

<400>  11

Gly Tyr Tyr Met His
1               5


<210>  12
<211>  11
<212>  PRT
<213>  Artificial

<220>
<223>  light chain CDR3L, <ANG-2> Ang2i_LC10 variant

<400>  12

Gln Val Trp Asp Ser Ser Ser Asp His Trp Val
1               5                   10


<210>  13
<211>  7
<212>  PRT
<213>  Artificial

<220>
<223>  light chain CDR2L, <ANG-2> Ang2i_LC10 variant

<400>  13

Asp Asp Ser Asp Arg Pro Ser
1               5


<210>  14
<211>  11
<212>  PRT
<213>  Artificial

<220>
<223>  light chain CDR1L, <ANG-2> Ang2i_LC10 variant

<400>  14
```

```
Gly Gly Asn Asn Ile Gly Ser Lys Ser Val His
1               5                   10


<210>  15
<211>  129
<212>  PRT
<213>  Artificial

<220>
<223>  heavy chain variable domain VH, <ANG-2> Ang2i_LC10 variant

<400>  15

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30


Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60


Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            100                 105                 110


Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        115                 120                 125


Ser



<210>  16
<211>  110
<212>  PRT
<213>  Artificial

<220>
<223>  light chain variable domain VL, <ANG-2> Ang2i_LC10 variant

<400>  16

Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1               5                   10                  15


Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30
```

```
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45


Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60


Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80


Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95


Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
            100                 105                 110


<210>  17
<211>  191
<212>  PRT
<213>  Homo sapiens

<400>  17

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1               5                   10                  15


Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
            20                  25                  30


Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
        35                  40                  45


Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60


Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65                  70                  75                  80


Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
                85                  90                  95


Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110


Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115                 120                 125


Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
    130                 135                 140


Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
145                 150                 155                 160


Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
```

```
                165                 170                 175


Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
            180                 185                 190


<210>  18
<211>  496
<212>  PRT
<213>  Homo sapiens

<400>  18

Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala
1               5                   10                  15


Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys
            20                  25                  30


Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro
        35                  40                  45


Glu Met Asp Asn Cys Arg Ser Ser Ser Ser Pro Tyr Val Ser Asn Ala
    50                  55                  60


Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Asp Ser Val Gln Arg Leu
65                  70                  75                  80


Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp Leu Met Lys
                85                  90                  95


Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Val Glu Ile
            100                 105                 110


Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile Glu Ile Gly
        115                 120                 125


Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
    130                 135                 140


Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu
145                 150                 155                 160


Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln Ile Leu Asp
                165                 170                 175


Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser Phe Leu Glu
            180                 185                 190


Lys Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln Leu Gln Ser
        195                 200                 205


Ile Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser Lys Gln Asn
    210                 215                 220
```

```
Ser Ile Ile Glu Glu Leu Glu Lys Lys Ile Val Thr Ala Thr Val Asn
225                 230                 235                 240

Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu Thr Val Asn
                245                 250                 255

Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Pro Thr
            260                 265                 270

Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Glu Val Phe
        275                 280                 285

Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe Pro Asn
    290                 295                 300

Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly Gly Gly
305                 310                 315                 320

Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp Phe Gln
                325                 330                 335

Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu
            340                 345                 350

Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg
        355                 360                 365

Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr
    370                 375                 380

Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg
385                 390                 395                 400

Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile
                405                 410                 415

Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys
            420                 425                 430

Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp
        435                 440                 445

Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln
    450                 455                 460

Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser
465                 470                 475                 480

Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe
                485                 490                 495
```

```
<210>  19
<211>  498
<212>  PRT
<213>  Homo sapiens

<400>  19

Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His
1               5                   10                  15


Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg
            20                  25                  30


Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro
        35                  40                  45


Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr
    50                  55                  60


Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser
65                  70                  75                  80


Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp
                85                  90                  95


Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met
            100                 105                 110


Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu
        115                 120                 125


Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys
    130                 135                 140


Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu
145                 150                 155                 160


Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln
                165                 170                 175


Leu Leu Gln Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser
            180                 185                 190


Leu Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu
        195                 200                 205


Leu Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr
    210                 215                 220


Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala
225                 230                 235                 240
```

```
Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp
                245                 250                 255


Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu
            260                 265                 270


Lys Gly Gly Lys Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp
        275                 280                 285


Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile
    290                 295                 300


Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn
305                 310                 315                 320


Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp
                325                 330                 335


Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser
            340                 345                 350


Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln
        355                 360                 365


Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg
    370                 375                 380


Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn
385                 390                 395                 400


Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser
                405                 410                 415


Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn
            420                 425                 430


Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp
        435                 440                 445


Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala
    450                 455                 460


Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys
465                 470                 475                 480


Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu
                485                 490                 495


Asp Phe
```

```
<210>  20
<211>  1124
<212>  PRT
<213>  Homo sapiens

<400>  20

Met Asp Ser Leu Ala Ser Leu Val Leu Cys Gly Val Ser Leu Leu Leu
1               5                   10                  15


Ser Gly Thr Val Glu Gly Ala Met Asp Leu Ile Leu Ile Asn Ser Leu
            20                  25                  30


Pro Leu Val Ser Asp Ala Glu Thr Ser Leu Thr Cys Ile Ala Ser Gly
        35                  40                  45


Trp Arg Pro His Glu Pro Ile Thr Ile Gly Arg Asp Phe Glu Ala Leu
    50                  55                  60


Met Asn Gln His Gln Asp Pro Leu Glu Val Thr Gln Asp Val Thr Arg
65                  70                  75                  80


Glu Trp Ala Lys Lys Val Val Trp Lys Arg Glu Lys Ala Ser Lys Ile
                85                  90                  95


Asn Gly Ala Tyr Phe Cys Glu Gly Arg Val Arg Gly Glu Ala Ile Arg
            100                 105                 110


Ile Arg Thr Met Lys Met Arg Gln Gln Ala Ser Phe Leu Pro Ala Thr
        115                 120                 125


Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val Asn Ile Ser Phe Lys
    130                 135                 140


Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly Ser
145                 150                 155                 160


Phe Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val
                165                 170                 175


His Leu Pro His Ala Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg
            180                 185                 190


Tyr Ile Gly Gly Asn Leu Phe Thr Ser Ala Phe Thr Arg Leu Ile Val
        195                 200                 205


Arg Arg Cys Glu Ala Gln Lys Trp Gly Pro Glu Cys Asn His Leu Cys
    210                 215                 220


Thr Ala Cys Met Asn Asn Gly Val Cys His Glu Asp Thr Gly Glu Cys
225                 230                 235                 240
```

```
Ile Cys Pro Pro Gly Phe Met Gly Arg Thr Cys Glu Lys Ala Cys Glu
                245                 250                 255

Leu His Thr Phe Gly Arg Thr Cys Lys Glu Arg Cys Ser Gly Gln Glu
            260                 265                 270

Gly Cys Lys Ser Tyr Val Phe Cys Leu Pro Asp Pro Tyr Gly Cys Ser
        275                 280                 285

Cys Ala Thr Gly Trp Lys Gly Leu Gln Cys Asn Glu Ala Cys His Pro
    290                 295                 300

Gly Phe Tyr Gly Pro Asp Cys Lys Leu Arg Cys Ser Cys Asn Asn Gly
305                 310                 315                 320

Glu Met Cys Asp Arg Phe Gln Gly Cys Leu Cys Ser Pro Gly Trp Gln
                325                 330                 335

Gly Leu Gln Cys Glu Arg Glu Gly Ile Pro Arg Met Thr Pro Lys Ile
            340                 345                 350

Val Asp Leu Pro Asp His Ile Glu Val Asn Ser Gly Lys Phe Asn Pro
        355                 360                 365

Ile Cys Lys Ala Ser Gly Trp Pro Leu Pro Thr Asn Glu Glu Met Thr
    370                 375                 380

Leu Val Lys Pro Asp Gly Thr Val Leu His Pro Lys Asp Phe Asn His
385                 390                 395                 400

Thr Asp His Phe Ser Val Ala Ile Phe Thr Ile His Arg Ile Leu Pro
                405                 410                 415

Pro Asp Ser Gly Val Trp Val Cys Ser Val Asn Thr Val Ala Gly Met
            420                 425                 430

Val Glu Lys Pro Phe Asn Ile Ser Val Lys Val Leu Pro Lys Pro Leu
        435                 440                 445

Asn Ala Pro Asn Val Ile Asp Thr Gly His Asn Phe Ala Val Ile Asn
    450                 455                 460

Ile Ser Ser Glu Pro Tyr Phe Gly Asp Gly Pro Ile Lys Ser Lys Lys
465                 470                 475                 480

Leu Leu Tyr Lys Pro Val Asn His Tyr Glu Ala Trp Gln His Ile Gln
                485                 490                 495

Val Thr Asn Glu Ile Val Thr Leu Asn Tyr Leu Glu Pro Arg Thr Glu
            500                 505                 510
```

```
Tyr Glu Leu Cys Val Gln Leu Val Arg Arg Gly Glu Gly Gly Glu Gly
        515                 520                 525


His Pro Gly Pro Val Arg Arg Phe Thr Thr Ala Ser Ile Gly Leu Pro
    530                 535                 540


Pro Pro Arg Gly Leu Asn Leu Leu Pro Lys Ser Gln Thr Thr Leu Asn
545                 550                 555                 560


Leu Thr Trp Gln Pro Ile Phe Pro Ser Ser Glu Asp Asp Phe Tyr Val
                565                 570                 575


Glu Val Glu Arg Arg Ser Val Gln Lys Ser Asp Gln Gln Asn Ile Lys
            580                 585                 590


Val Pro Gly Asn Leu Thr Ser Val Leu Leu Asn Asn Leu His Pro Arg
        595                 600                 605


Glu Gln Tyr Val Val Arg Ala Arg Val Asn Thr Lys Ala Gln Gly Glu
    610                 615                 620


Trp Ser Glu Asp Leu Thr Ala Trp Thr Leu Ser Asp Ile Leu Pro Pro
625                 630                 635                 640


Gln Pro Glu Asn Ile Lys Ile Ser Asn Ile Thr His Ser Ser Ala Val
                645                 650                 655


Ile Ser Trp Thr Ile Leu Asp Gly Tyr Ser Ile Ser Ser Ile Thr Ile
            660                 665                 670


Arg Tyr Lys Val Gln Gly Lys Asn Glu Asp Gln His Val Asp Val Lys
        675                 680                 685


Ile Lys Asn Ala Thr Ile Thr Gln Tyr Gln Leu Lys Gly Leu Glu Pro
    690                 695                 700


Glu Thr Ala Tyr Gln Val Asp Ile Phe Ala Glu Asn Asn Ile Gly Ser
705                 710                 715                 720


Ser Asn Pro Ala Phe Ser His Glu Leu Val Thr Leu Pro Glu Ser Gln
                725                 730                 735


Ala Pro Ala Asp Leu Gly Gly Gly Lys Met Leu Leu Ile Ala Ile Leu
            740                 745                 750


Gly Ser Ala Gly Met Thr Cys Leu Thr Val Leu Leu Ala Phe Leu Ile
        755                 760                 765


Ile Leu Gln Leu Lys Arg Ala Asn Val Gln Arg Arg Met Ala Gln Ala
    770                 775                 780
```

```
Phe Gln Asn Val Arg Glu Glu Pro Ala Val Gln Phe Asn Ser Gly Thr
785                 790                 795                 800


Leu Ala Leu Asn Arg Lys Val Lys Asn Asn Pro Asp Pro Thr Ile Tyr
                805                 810                 815


Pro Val Leu Asp Trp Asn Asp Ile Lys Phe Gln Asp Val Ile Gly Glu
            820                 825                 830


Gly Asn Phe Gly Gln Val Leu Lys Ala Arg Ile Lys Lys Asp Gly Leu
        835                 840                 845


Arg Met Asp Ala Ala Ile Lys Arg Met Lys Glu Tyr Ala Ser Lys Asp
    850                 855                 860


Asp His Arg Asp Phe Ala Gly Glu Leu Glu Val Leu Cys Lys Leu Gly
865                 870                 875                 880


His His Pro Asn Ile Ile Asn Leu Leu Gly Ala Cys Glu His Arg Gly
                885                 890                 895


Tyr Leu Tyr Leu Ala Ile Glu Tyr Ala Pro His Gly Asn Leu Leu Asp
            900                 905                 910


Phe Leu Arg Lys Ser Arg Val Leu Glu Thr Asp Pro Ala Phe Ala Ile
        915                 920                 925


Ala Asn Ser Thr Ala Ser Thr Leu Ser Ser Gln Gln Leu Leu His Phe
    930                 935                 940


Ala Ala Asp Val Ala Arg Gly Met Asp Tyr Leu Ser Gln Lys Gln Phe
945                 950                 955                 960


Ile His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Gly Glu Asn Tyr
                965                 970                 975


Val Ala Lys Ile Ala Asp Phe Gly Leu Ser Arg Gly Gln Glu Val Tyr
            980                 985                 990


Val Lys Lys Thr Met Gly Arg Leu  Pro Val Arg Trp Met  Ala Ile Glu
        995                 1000                 1005


Ser Leu  Asn Tyr Ser Val Tyr  Thr Thr Asn Ser Asp  Val Trp Ser
    1010                 1015                 1020


Tyr Gly  Val Leu Leu Trp Glu  Ile Val Ser Leu Gly  Gly Thr Pro
    1025                 1030                 1035


Tyr Cys  Gly Met Thr Cys Ala  Glu Leu Tyr Glu Lys  Leu Pro Gln
    1040                 1045                 1050
```

```
Gly Tyr  Arg Leu Glu Lys Pro  Leu Asn Cys Asp Asp  Glu Val Tyr
    1055                 1060                 1065


Asp Leu  Met Arg Gln Cys Trp  Arg Glu Lys Pro Tyr  Glu Arg Pro
    1070                 1075                 1080


Ser Phe  Ala Gln Ile Leu Val  Ser Leu Asn Arg Met  Leu Glu Glu
    1085                 1090                 1095


Arg Lys  Thr Tyr Val Asn Thr  Thr Leu Tyr Glu Lys  Phe Thr Tyr
    1100                 1105                 1110


Ala Gly  Ile Asp Cys Ser Ala  Glu Glu Ala Ala
    1115                 1120


<210>  21
<211>  453
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 1 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations
       (VEGFang2-0012)

<400>  21

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr
            20                  25                  30


Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60


Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
            100                 105                 110


Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125


Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140


Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
```

```
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ala Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu Ala Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
```

```
            420                 425                 430


Val Met His Glu Ala Leu His Asn Ala Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445


Leu Ser Pro Gly Lys
    450


<210>  22
<211>  463
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 2 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations
       (VEGFang2-0012)

<400>  22

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30


Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60


Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            100                 105                 110


Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        115                 120                 125


Ser Ala Ser Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
    130                 135                 140


Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145                 150                 155                 160


Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
                165                 170                 175


Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
            180                 185                 190
```

```
Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
        195                 200                 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
    210                 215                 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Asp Lys Thr His
225                 230                 235                 240

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
                245                 250                 255

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ala Ser Arg Thr
            260                 265                 270

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        275                 280                 285

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    290                 295                 300

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
305                 310                 315                 320

Val Leu Thr Val Leu Ala Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                325                 330                 335

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
            340                 345                 350

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro
        355                 360                 365

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala
    370                 375                 380

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385                 390                 395                 400

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                405                 410                 415

Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg
            420                 425                 430

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        435                 440                 445

His Asn Ala Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455                 460
```

```
<210>  23
<211>  214
<212>  PRT
<213>  Artificial

<220>
<223>  Light chain 1 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations
       (VEGFang2-0012)

<400>  23

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30


Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45


Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110


Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125


Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140


Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160


Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175


Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190


Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205


Phe Asn Arg Gly Glu Cys
    210
```

```
<210>  24
<211>  213
<212>  PRT
<213>  Artificial

<220>
<223>  Light chain 2 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations
       (VEGF-Ang2-0012)

<400>  24

Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1               5                   10                  15


Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30


His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45


Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60


Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80


Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95


Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Ser Ala Ser
            100                 105                 110


Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
        115                 120                 125


Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
    130                 135                 140


Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
145                 150                 155                 160


His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
                165                 170                 175


Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
            180                 185                 190


Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
        195                 200                 205


Glu Pro Lys Ser Cys
    210
```

<210>  25
<211>  453
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 1 of  <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations
       and P329G LALA mutations (VEGFang2-0016)

<400>  25

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala

```
225                 230                 235                 240

Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ala Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu Ala Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430

Val Met His Glu Ala Leu His Asn Ala Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445

Leu Ser Pro Gly Lys
    450


<210>  26
<211>  463
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 2 of  <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations
       and P329G LALA mutations (VEGFang2-0016)
```

```
<400>  26

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30


Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60


Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            100                 105                 110


Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        115                 120                 125


Ser Ala Ser Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
    130                 135                 140


Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145                 150                 155                 160


Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
                165                 170                 175


Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
            180                 185                 190


Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
        195                 200                 205


Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
    210                 215                 220


Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Asp Lys Thr His
225                 230                 235                 240


Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
                245                 250                 255


Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ala Ser Arg Thr
            260                 265                 270
```

```
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        275                 280                 285


Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    290                 295                 300


Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
305                 310                 315                 320


Val Leu Thr Val Leu Ala Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                325                 330                 335


Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile
            340                 345                 350


Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro
        355                 360                 365


Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala
    370                 375                 380


Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385                 390                 395                 400


Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                405                 410                 415


Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg
            420                 425                 430


Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        435                 440                 445


His Asn Ala Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455                 460


<210>  27
<211>  214
<212>  PRT
<213>  Artificial

<220>
<223>  Light chain 1 of  <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations
       and P329G LALA mutations (VEGFang2-0016)

<400>  27

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30
```

```
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45


Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110


Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125


Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140


Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160


Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175


Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190


Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205


Phe Asn Arg Gly Glu Cys
    210


<210>  28
<211>  213
<212>  PRT
<213>  Artificial

<220>
<223>  Light chain 2 of  <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations
       and P329G LALA mutations (VEGFang2-0016)

<400>  28

Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1               5                   10                  15


Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30
```

```
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45


Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60


Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80


Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95


Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Ser Ala Ser
            100                 105                 110


Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
        115                 120                 125


Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
    130                 135                 140


Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
145                 150                 155                 160


His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
                165                 170                 175


Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
            180                 185                 190


Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
        195                 200                 205


Glu Pro Lys Ser Cys
    210


<210>  29
<211>  450
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 1 of <VEGF-ANG-2> CrossMAb IgG4 with AAA mutations
       and with SPLE mutations

<400>  29

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr
            20                  25                  30


Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
```

```
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val
        195                 200                 205

Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys
    210                 215                 220

Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ala
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu
            260                 265                 270

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu Ala Gln Asp Trp Leu Asn Gly Lys
```

```
305                 310                 315                 320


Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu
                325                 330                 335


Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys
            340                 345                 350


Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365


Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380


Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400


Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Arg Leu Thr Val Asp
                405                 410                 415


Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430


Glu Ala Leu His Asn Ala Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu
        435                 440                 445


Gly Lys
    450


<210>  30
<211>  460
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 2 of <VEGF-ANG-2> CrossMAb IgG4 with AAA mutations
       and with SPLE mutations

<400>  30

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30


Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60


Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80
```

```
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            100                 105                 110

Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        115                 120                 125

Ser Ala Ser Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
    130                 135                 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145                 150                 155                 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
                165                 170                 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
            180                 185                 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
        195                 200                 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
    210                 215                 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Pro Pro Cys Pro
225                 230                 235                 240

Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val Phe Leu Phe
                245                 250                 255

Pro Pro Lys Pro Lys Asp Thr Leu Met Ala Ser Arg Thr Pro Glu Val
            260                 265                 270

Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe
        275                 280                 285

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
    290                 295                 300

Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
305                 310                 315                 320

Val Leu Ala Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
                325                 330                 335

Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala
            340                 345                 350
```

```
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Gln
        355                 360                 365


Glu Glu Met Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly
    370                 375                 380


Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
385                 390                 395                 400


Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
                405                 410                 415


Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu
            420                 425                 430


Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Ala
        435                 440                 445


Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
    450                 455                 460


<210>  31
<211>  214
<212>  PRT
<213>  Artificial

<220>
<223>  Light chain 1 of <VEGF-ANG-2> CrossMAb IgG4 with AAA mutations
       and with SPLE mutations

<400>  31

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30


Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45


Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110
```

```
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125


Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140


Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160


Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175


Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190


Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205


Phe Asn Arg Gly Glu Cys
    210


<210>  32
<211>  213
<212>  PRT
<213>  Artificial

<220>
<223>  Light chain 2 of <VEGF-ANG-2> CrossMAb IgG4 with AAA mutations
       and with SPLE mutations

<400>  32

Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1               5                   10                  15


Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30


His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45


Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60


Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80


Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95


Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Ser Ala Ser
            100                 105                 110
```

```
Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr
        115                 120                 125


Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
    130                 135                 140


Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
145                 150                 155                 160


His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
                165                 170                 175


Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr
            180                 185                 190


Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val
        195                 200                 205


Glu Ser Lys Tyr Gly
    210


<210>  33
<211>  453
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 1 of <VEGF-ANG-2> OAscFab IgG1 with AAA mutations

<400>  33

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr
            20                  25                  30


Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60


Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
            100                 105                 110


Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125
```

```
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ala Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu Ala Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400
```

```
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu
                405                 410                 415


Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430


Val Met His Glu Ala Leu His Asn Ala Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445


Leu Ser Pro Gly Lys
    450


<210>  34
<211>  705
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 2 of <VEGF-ANG-2> OAscFab IgG1 with AAA mutations

<400>  34

Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1               5                   10                  15


Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30


His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45


Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60


Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80


Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95


Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro Lys
            100                 105                 110


Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Gln
        115                 120                 125


Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro Gly
    130                 135                 140


Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala Gly
145                 150                 155                 160
```

```
Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala
                165                 170                 175

Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg Ser
            180                 185                 190

Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val
        195                 200                 205

Ala Pro Thr Glu Cys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    210                 215                 220

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
225                 230                 235                 240

Gly Gly Gly Ser Gly Gly Gln Val Gln Leu Val Glu Ser Gly Ala Glu
                245                 250                 255

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
            260                 265                 270

Tyr Thr Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly
        275                 280                 285

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
    290                 295                 300

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
305                 310                 315                 320

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
                325                 330                 335

Thr Ala Val Tyr Tyr Cys Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp
            340                 345                 350

Ser Ser Gly Tyr Tyr Tyr Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly
        355                 360                 365

Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
    370                 375                 380

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
385                 390                 395                 400

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
                405                 410                 415

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
            420                 425                 430
```

```
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
        435                 440                 445


Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
    450                 455                 460


Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
465                 470                 475                 480


Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
                485                 490                 495


Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ala Ser
            500                 505                 510


Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
        515                 520                 525


Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
    530                 535                 540


Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
545                 550                 555                 560


Val Ser Val Leu Thr Val Leu Ala Gln Asp Trp Leu Asn Gly Lys Glu
                565                 570                 575


Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
            580                 585                 590


Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
        595                 600                 605


Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp
    610                 615                 620


Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
625                 630                 635                 640


Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
                645                 650                 655


Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
            660                 665                 670


Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
        675                 680                 685


Ala Leu His Asn Ala Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
    690                 695                 700
```

```
Lys
705


<210>  35
<211>  214
<212>  PRT
<213>  Artificial

<220>
<223>  Light chain 1 of <VEGF-ANG-2> OAscFab IgG1 with AAA mutations

<400>  35

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30


Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45


Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110


Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125


Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140


Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160


Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175


Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190


Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205


Phe Asn Arg Gly Glu Cys
    210
```

```
<210>  36
<211>  450
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 1 of <VEGF-ANG-2> OAscFab IgG4 with AAA mutations
       and with SPLE mutations

<400>  36

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr
            20                  25                  30


Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60


Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
            100                 105                 110


Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125


Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser
    130                 135                 140


Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160


Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175


Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190


Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val
        195                 200                 205


Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys
    210                 215                 220
```

```
Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly
225                 230                 235                 240


Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ala
                245                 250                 255


Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu
            260                 265                 270


Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285


Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg
    290                 295                 300


Val Val Ser Val Leu Thr Val Leu Ala Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320


Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu
                325                 330                 335


Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys
            340                 345                 350


Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365


Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380


Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400


Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Arg Leu Thr Val Asp
                405                 410                 415


Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430


Glu Ala Leu His Asn Ala Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu
        435                 440                 445


Gly Lys
    450


<210>  37
<211>  702
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 2 of <VEGF-ANG-2> OAscFab IgG4 with AAA mutations and
```

```
       with SPLE mutations

<400>  37

Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1               5                   10                  15


Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30


His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45


Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60


Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80


Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95


Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro Lys
            100                 105                 110


Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Gln
        115                 120                 125


Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro Gly
    130                 135                 140


Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala Gly
145                 150                 155                 160


Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala
                165                 170                 175


Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg Ser
            180                 185                 190


Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val
        195                 200                 205


Ala Pro Thr Glu Cys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    210                 215                 220


Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
225                 230                 235                 240


Gly Gly Gly Ser Gly Gly Gln Val Gln Leu Val Glu Ser Gly Ala Glu
                245                 250                 255
```

```
Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
            260                 265                 270


Tyr Thr Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly
        275                 280                 285


Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
    290                 295                 300


Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
305                 310                 315                 320


Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
                325                 330                 335


Thr Ala Val Tyr Tyr Cys Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp
            340                 345                 350


Ser Ser Gly Tyr Tyr Tyr Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly
        355                 360                 365


Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
    370                 375                 380


Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
385                 390                 395                 400


Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
                405                 410                 415


Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
            420                 425                 430


Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
        435                 440                 445


Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro
    450                 455                 460


Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
465                 470                 475                 480


Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val Phe
                485                 490                 495


Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ala Ser Arg Thr Pro
            500                 505                 510


Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val
        515                 520                 525
```

```
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
    530                 535                 540


Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val
545                 550                 555                 560


Leu Thr Val Leu Ala Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
                565                 570                 575


Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser
            580                 585                 590


Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
        595                 600                 605


Cys Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val
    610                 615                 620


Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
625                 630                 635                 640


Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
                645                 650                 655


Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp
            660                 665                 670


Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
        675                 680                 685


Asn Ala Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
    690                 695                 700


<210>  38
<211>  214
<212>  PRT
<213>  Artificial

<220>
<223>  Light chain 1 of <VEGF-ANG-2> OAscFab IgG4 with AAA mutations
       and with SPLE mutations

<400>  38

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30


Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45


Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
```

```
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110


Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125


Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140


Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160


Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175


Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190


Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205


Phe Asn Arg Gly Glu Cys
    210


<210>  39
<211>  453
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 1 of <VEGF-ANG-2> CrossMAb IgG1 wild type (without
       AAA mutations) (VEGFang2-0201)

<400>  39

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr
            20                  25                  30


Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60
```

```
Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
            100                 105                 110


Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125


Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140


Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160


Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175


Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190


Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205


Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220


Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240


Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255


Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270


Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285


Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300


Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320


Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335
```

```
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350


Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln
        355                 360                 365


Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380


Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400


Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415


Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430


Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445


Leu Ser Pro Gly Lys
    450


<210>  40
<211>  463
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 2 of <VEGF-ANG-2> CrossMAb IgG1 wild type (without
       AAA mutations) (VEGFang2-0201)

<400>  40

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30


Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60


Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
```

```
Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            100                 105                 110

Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        115                 120                 125

Ser Ala Ser Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
    130                 135                 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145                 150                 155                 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
                165                 170                 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
            180                 185                 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
        195                 200                 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
    210                 215                 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Asp Lys Thr His
225                 230                 235                 240

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
                245                 250                 255

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            260                 265                 270

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        275                 280                 285

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    290                 295                 300

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
305                 310                 315                 320

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                325                 330                 335

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
            340                 345                 350

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro
        355                 360                 365
```

```
Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala
    370                 375                 380


Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385                 390                 395                 400


Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                405                 410                 415


Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg
            420                 425                 430


Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        435                 440                 445


His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455                 460


<210>  41
<211>  214
<212>  PRT
<213>  Artificial

<220>
<223>  Light chain 1 of <VEGF-ANG-2> CrossMAb IgG1 wild type ( without
       AAA mutations) (VEGFang2-0201)

<400>  41

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30


Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45


Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110


Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125
```

```
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140


Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160


Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175


Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190


Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205


Phe Asn Arg Gly Glu Cys
    210


<210>  42
<211>  213
<212>  PRT
<213>  Artificial

<220>
<223>  Light chain 2 of <VEGF-ANG-2> CrossMAb IgG1 wild type (without
       AAA mutations) (VEGFang2-0201)

<400>  42

Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1               5                   10                  15


Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30


His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45


Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60


Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80


Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95


Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Ser Ala Ser
            100                 105                 110


Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
        115                 120                 125


Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
```

```
    130                 135                 140


Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
145                 150                 155                 160


His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
                165                 170                 175


Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
            180                 185                 190


Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
        195                 200                 205


Glu Pro Lys Ser Cys
    210


<210>  43
<211>  453
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 1 of <VEGF-ANG-2> CrossMAb IgG1  with P329G LALA
       mutations only (without AAA mutations) (VEGFang2-0015)

<400>  43

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr
            20                  25                  30


Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60


Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
            100                 105                 110


Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125


Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140
```

```
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala
225                 230                 235                 240

Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415
```

```
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430


Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445


Leu Ser Pro Gly Lys
    450


<210>  44
<211>  463
<212>  PRT
<213>  Artificial

<220>
<223>  Heavy chain 2 of <VEGF-ANG-2> CrossMAb IgG1 with P329G LALA
       mutations only (without AAA mutations) (VEGFang2-0015)

<400>  44

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30


Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60


Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            100                 105                 110


Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        115                 120                 125


Ser Ala Ser Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
    130                 135                 140


Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145                 150                 155                 160


Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
                165                 170                 175
```

```
Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
            180                 185                 190


Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
        195                 200                 205


Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
    210                 215                 220


Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Asp Lys Thr His
225                 230                 235                 240


Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
                245                 250                 255


Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            260                 265                 270


Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        275                 280                 285


Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    290                 295                 300


Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
305                 310                 315                 320


Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                325                 330                 335


Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile
            340                 345                 350


Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro
        355                 360                 365


Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala
    370                 375                 380


Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385                 390                 395                 400


Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                405                 410                 415


Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg
            420                 425                 430


Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        435                 440                 445
```

```
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455                 460


<210>  45
<211>  214
<212>  PRT
<213>  Artificial

<220>
<223>  Light chain 1 of <VEGF-ANG-2> CrossMAb IgG1 with P329G LALA
       mutations only (without AAA mutations) (VEGFang2-0015)

<400>  45

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30


Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45


Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110


Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125


Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140


Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160


Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175


Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190


Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205
```

```
Phe Asn Arg Gly Glu Cys
    210


<210>  46
<211>  213
<212>  PRT
<213>  Artificial

<220>
<223>  Light chain 2 of <VEGF-ANG-2> CrossMAb IgG1 with P329G LALA
       mutations only (without AAA mutations) (VEGFang2-0015)

<400>  46

Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1               5                   10                  15


Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30


His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45


Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60


Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80


Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95


Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Ser Ala Ser
            100                 105                 110


Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
        115                 120                 125


Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
    130                 135                 140


Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
145                 150                 155                 160


His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
                165                 170                 175


Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
            180                 185                 190


Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
        195                 200                 205


Glu Pro Lys Ser Cys
```

```
    210


<210>  47
<211>  107
<212>  PRT
<213>  Homo sapiens

<400>  47

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1               5                   10                  15


Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
            20                  25                  30


Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
        35                  40                  45


Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
    50                  55                  60


Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65                  70                  75                  80


Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
                85                  90                  95


Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105


<210>  48
<211>  105
<212>  PRT
<213>  homo sapiens

<400>  48

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
1               5                   10                  15


Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
            20                  25                  30


Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
        35                  40                  45


Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
    50                  55                  60


Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
65                  70                  75                  80


His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
                85                  90                  95
```

```
Lys Thr Val Ala Pro Thr Glu Cys Ser
            100                 105


<210>  49
<211>  330
<212>  PRT
<213>  Homo sapiens

<400>  49

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                   10                  15


Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30


Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45


Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60


Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80


Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95


Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110


Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125


Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140


Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160


Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175


Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190


His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205


Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220
```

```
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225                 230                 235                 240


Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255


Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270


Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285


Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300


Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320


Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330


<210>  50
<211>  327
<212>  PRT
<213>  Homo sapiens

<400>  50

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1               5                   10                  15


Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30


Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45


Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60


Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65                  70                  75                  80


Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95


Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro
            100                 105                 110


Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
        115                 120                 125


Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
```

```
    130                 135                 140


Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145                 150                 155                 160


Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
                165                 170                 175


Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
            180                 185                 190


Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
        195                 200                 205


Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
    210                 215                 220


Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225                 230                 235                 240


Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
                245                 250                 255


Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
            260                 265                 270


Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
        275                 280                 285


Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
    290                 295                 300


Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305                 310                 315                 320


Leu Ser Leu Ser Leu Gly Lys
                325
```