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(54) FC-REGION VARIANTS WITH MODIFIED FCRN- AND MAINTAINED PROTEIN A-BINDING PROPERTIES

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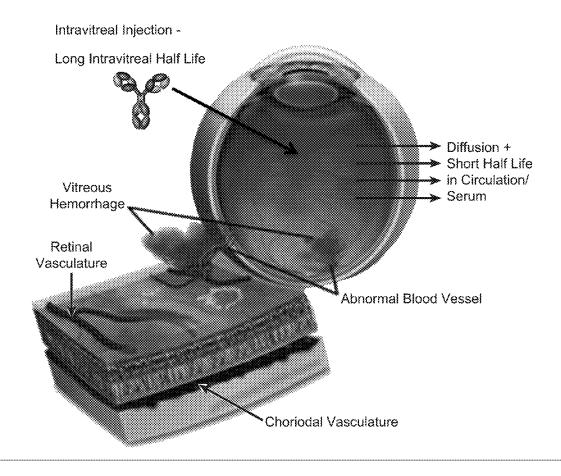
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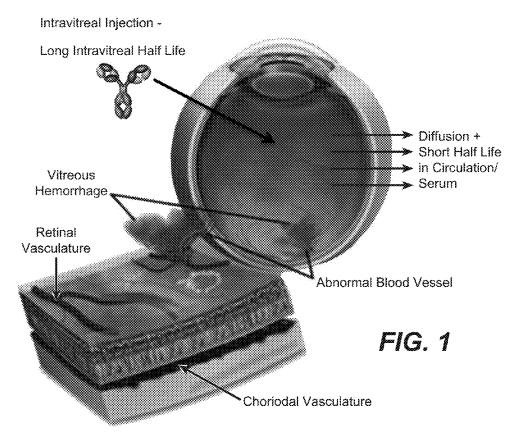
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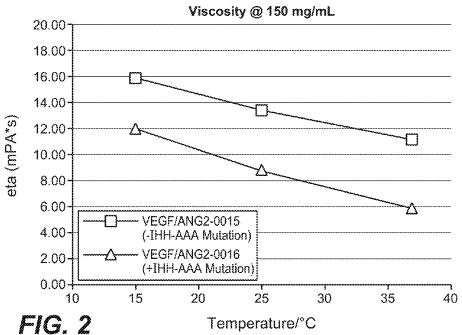
(57)ABSTRACT

Herein is reported a polypeptide comprising a first polypeptide and a second polypeptide each comprising in N-terminal to C-terminal direction at least a portion of an immunoglobulin hinge region, which comprises one or more cysteine residues, an immunoglobulin CH2-domain and an immunoglobulin CH3-domain, wherein

- i) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
- ii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
- iii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S.









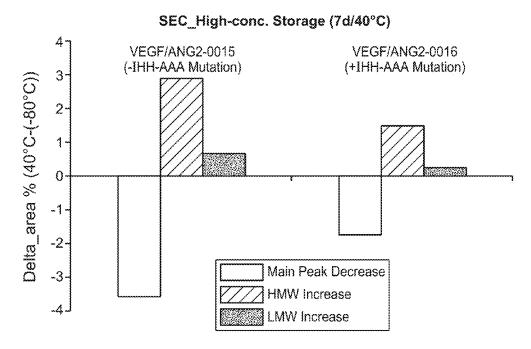
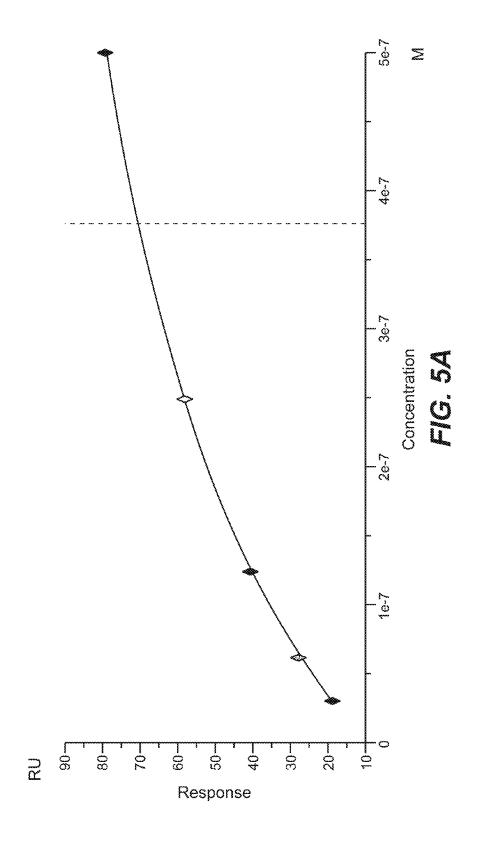
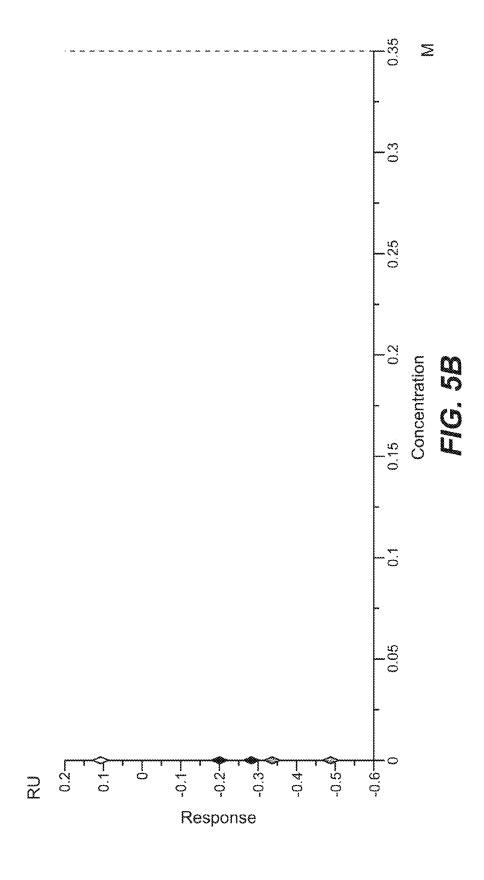
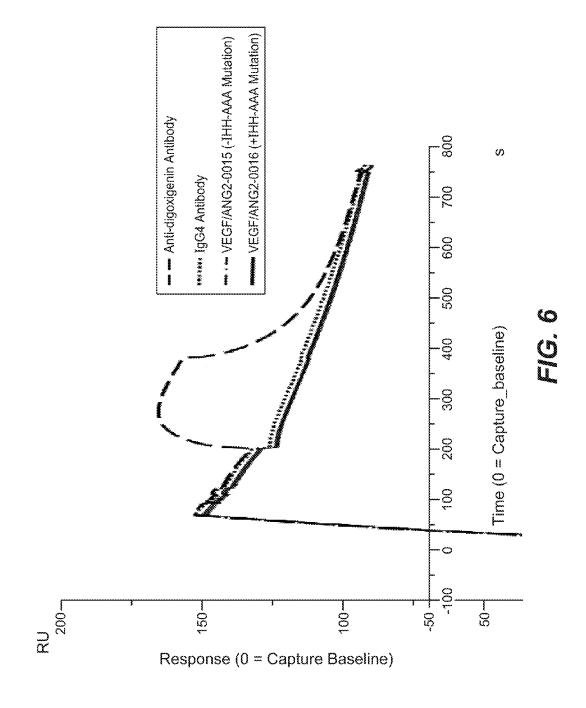
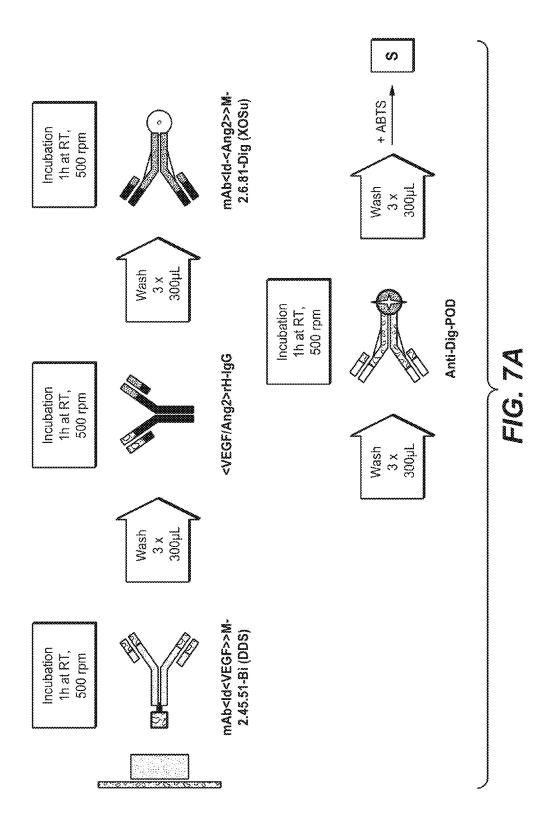


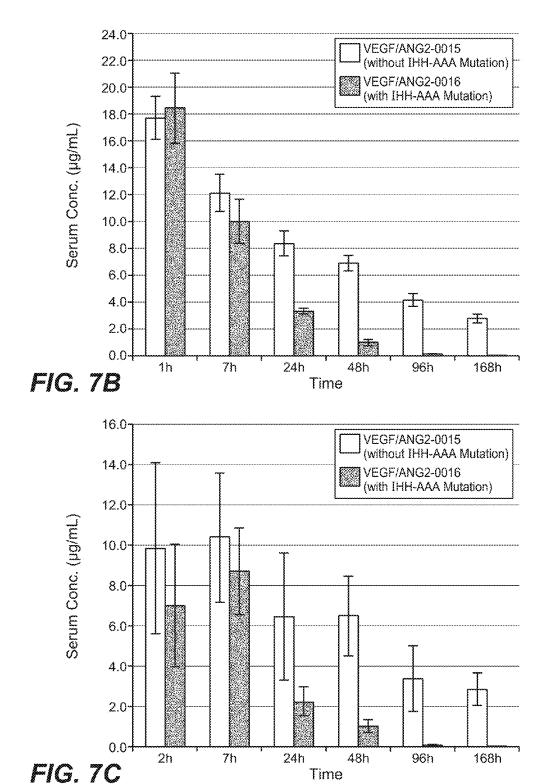
FIG. 4



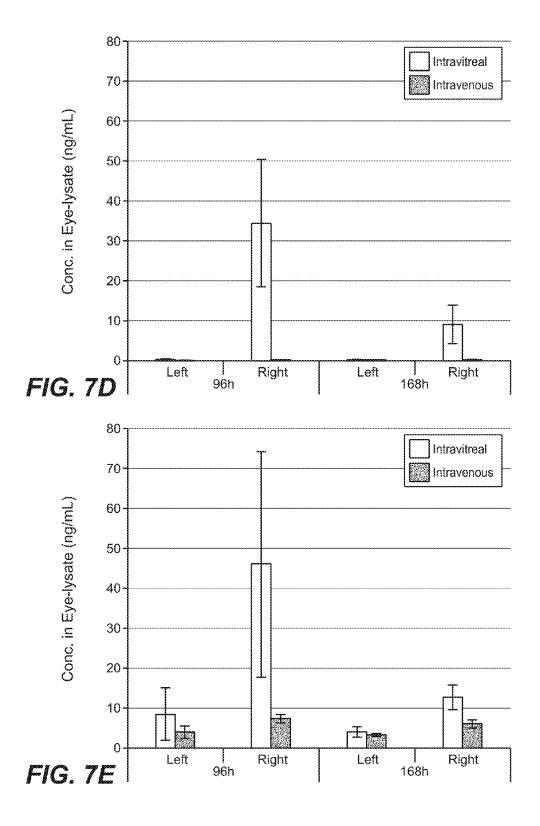


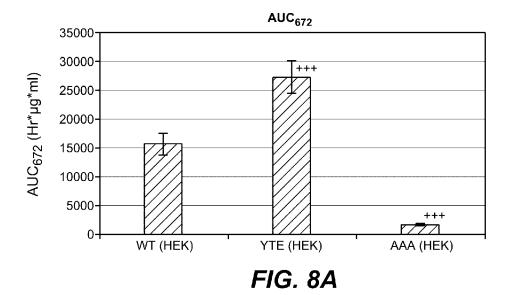


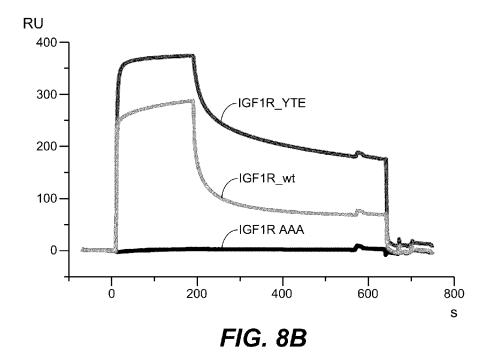


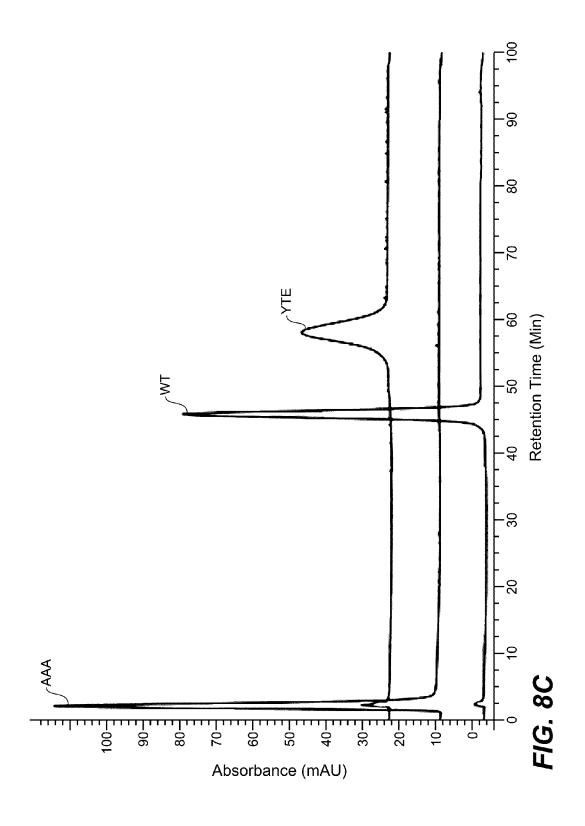


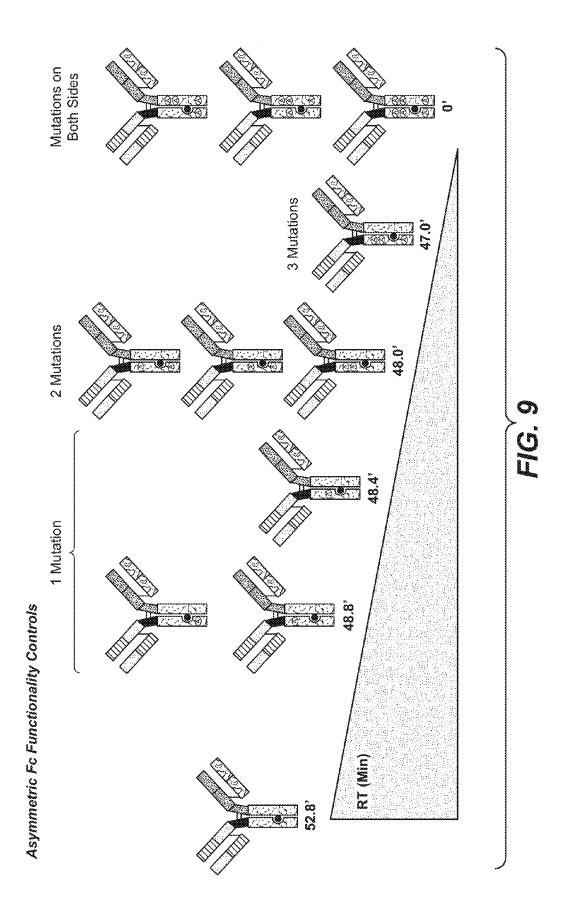
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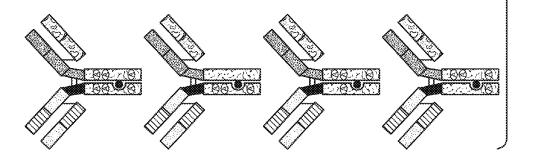




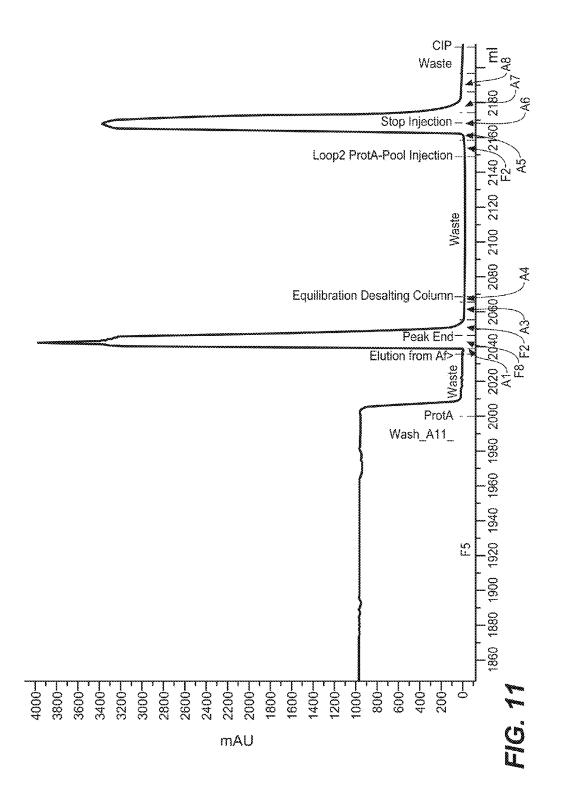


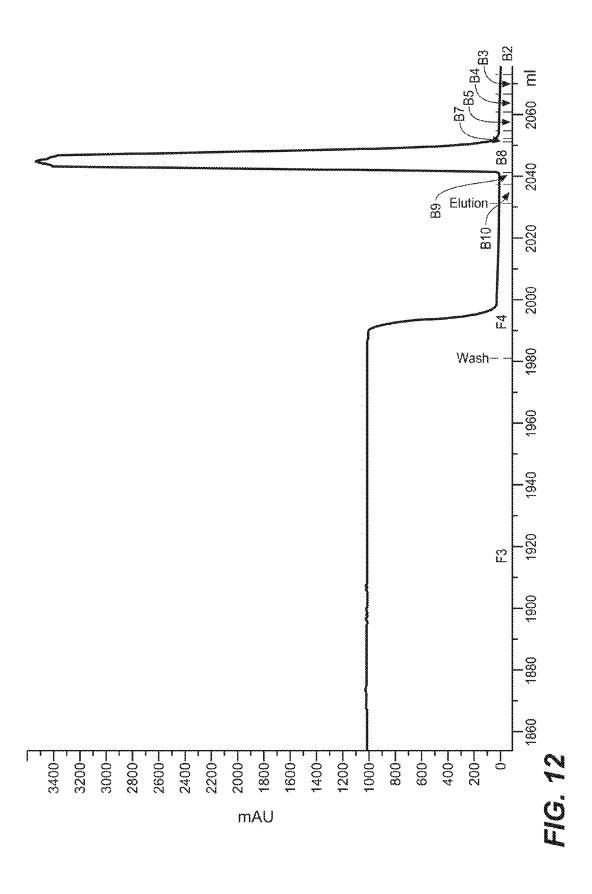


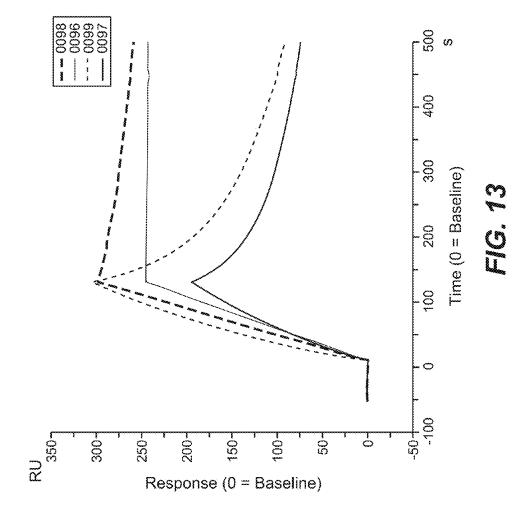
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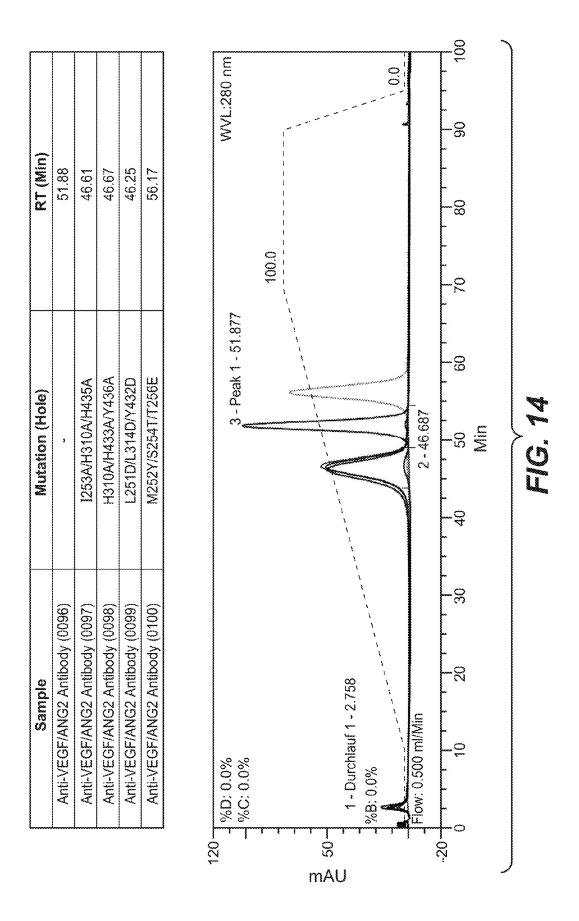


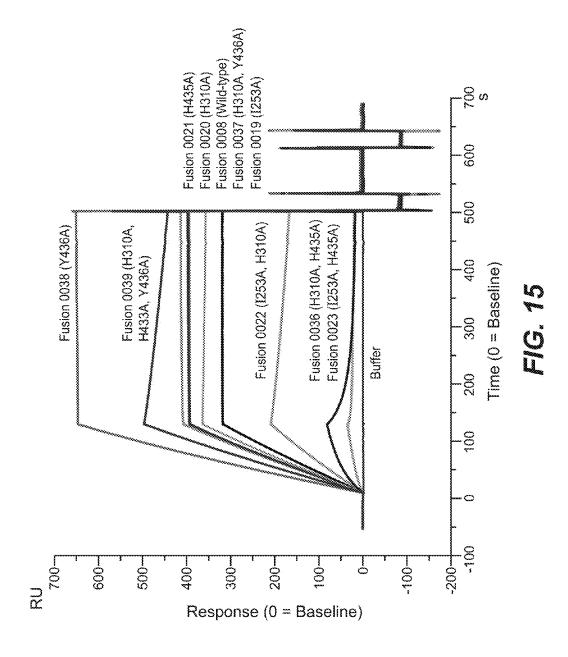
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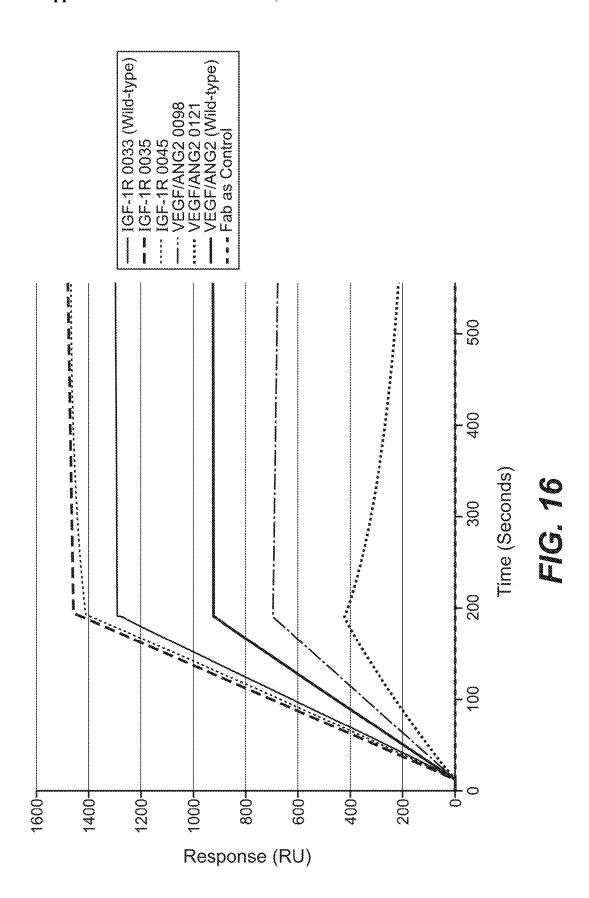


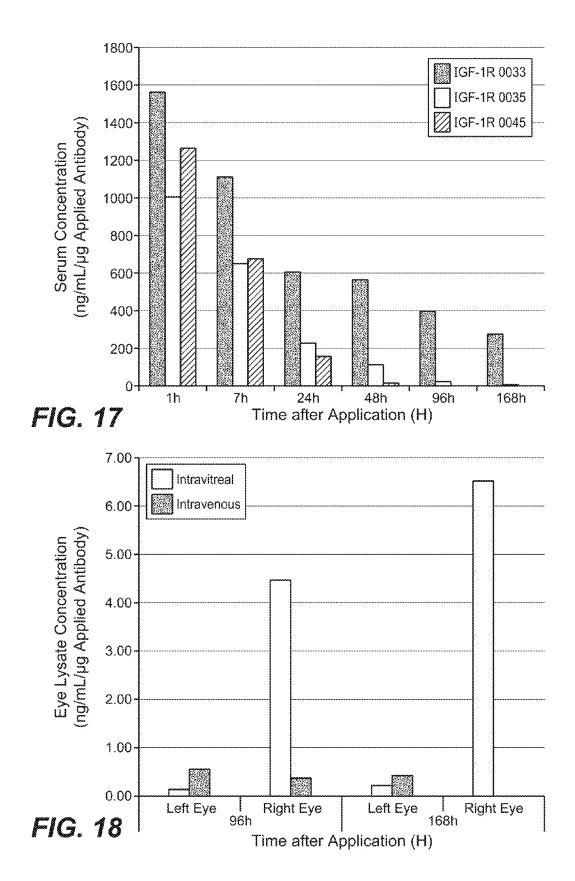


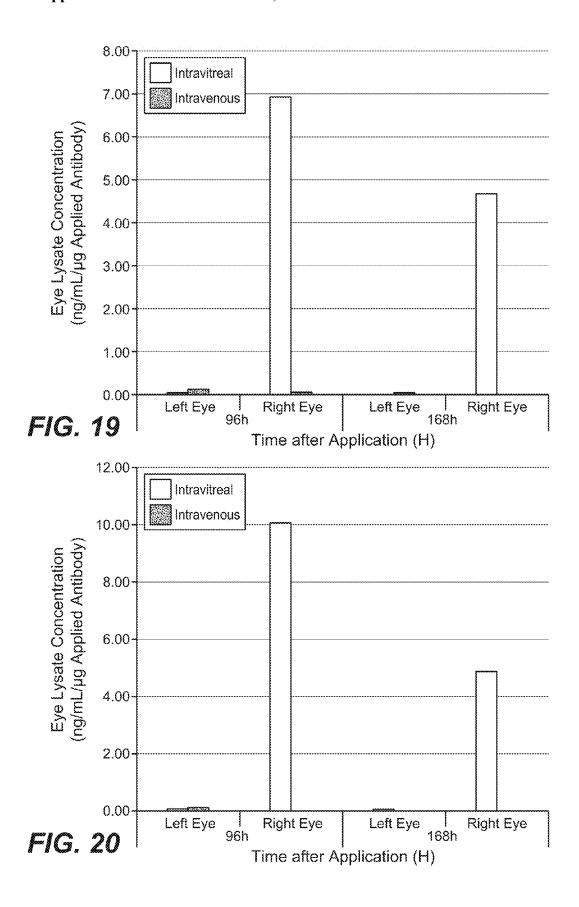












FC-REGION VARIANTS WITH MODIFIED FCRN- AND MAINTAINED PROTEIN A-BINDING PROPERTIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of International Patent Application No. PCT/EP2015/050426, having an international filing date of Jan. 12, 2015, the entire contents of which are incorporated herein by reference, and which claims benefit under 35 U.S.C. §119 to European Patent Application No. 14151320.0, filed on Jan. 15, 2014 and European Patent Application No. 14165923.5, filed on Apr. 25, 2014.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing submitted via EFS-Web and hereby incorporated by reference in its entirety. Said ASCII copy, created Jul. 13, 2016, is named P31953-US_SequenceListing.txt, 308,683 bytes in size.

BACKGROUND OF THE INVENTION

[0003] Herein are reported IgG Fc-regions that have been modified with respect to Fc-receptor binding without impairing their purification properties.

[0004] The demand for cost efficient production processes has led to the necessity of optimization of the downstream purification, including one or more affinity chromatography steps. Larger volumes to be processed and harder requirements for the cleaning-in-place (CIP) protocols are some of the features that need to be solved (Hober, S., J. Chrom. B. 848 (2007) 40-47).

[0005] The purification of monoclonal antibodies by means of selective Fc-region affinity ligands is the most promising methodology for the large-scale production of therapeutic monoclonal antibodies. In fact, this procedure does not require establishing any interaction with the antigen specific part of the antibody, i.e. the Fab domain, which is, thus, left intact and can retain its properties (see Salvalaglio, M., et al., J. Chrom. A 1216 (2009) 8678-8686).

[0006] Due to its selectiveness, an affinity-purification step is employed early in the purification chain and thereby the number of successive unit operations can be reduced (see Hober supra; MacLennan, J., Biotechnol. 13 (1995) 1180; Harakas, N. K., Bioprocess Technol. 18 (1994) 259).

[0007] The ligands most adopted to bind selectively IgG are Staphylococcal protein A and protein G, which are able to establish highly selective interactions with the Fc-region of most IgGs in a region known as "consensus binding site" (CBS) (DeLano, W. L., et al., Science 287 (2000) 1279), which is located at the hinge region between the CH2 and CH3 domains of the Fc-region.

[0008] Staphylococcal protein A (SPA) is a cell wall associated protein domain exposed on the surface of the Gram-positive bacterium *Staphylococcus aureus*. SPA has high affinity to IgG from various species, for instance human, rabbit and guinea pig IgG but only weak interaction with bovine and mouse IgG (see the following Table) (see Hober supra; Duhamel, R. C., et al., J. Immunol. Methods 31 (1979) 211; Björk, L. and Kronvall, G., Immunol. J. 133 (1984) 969; Richman, D. D., et al., J. Immunol. 128 (1982) 2300; Amersham Pharmacia Biotech, Handbook, Antibody Purification (2000)).

species	subclass	protein A binding
human	IgG1	++
	IgG2	++
	IgG3	
	IgG4	++
	IgA	variable
	IgD	_
	IgM	variable
rabbit	no distinction	++
guinea pig	IgG1	++
	IgG2	++
bovine		+
mouse	IgG1	+
	IgG2a	++
	IgG2b	+
	IgG3	+
	IgM	variable
chicken	IgY	-

++: strong binding/+: medium binding/-: weak or no interaction

[0009] The heavy chain hinge-region between the CH2 and CH3 domains of IgG is able to bind several proteins beyond protein A, such as the neonatal Fc receptor (FcRn) (see DeLano and Salvalaglio supra).

[0010] The SPA CBS comprehends a hydrophobic pocket on the surface of the antibody. The residues composing the IgG CBS are Ile 253, Ser 254, Met 252, Met 423, Tyr 326, His 435, Asn 434, His 433, Arg 255, and Glu 380 (numbering of the IgG heavy chain residues according to the Kabat EU index numbering system). The charged amino acids (Arg 255, Glu 380) are placed around a hydrophobic knob formed by Ile 253 and Ser 254. This (can) result in the establishment of polar and hydrophilic interactions (see Salvalaglio supra).

[0011] In general, the protein A-IgG interaction can be described using two main binding sites: the first is positioned in the heavy chain CH2 domain and is characterized by hydrophobic interactions between Phe 132, Leu 136, Ile 150 (of protein A) and the IgG hydrophobic knob constituted by Ile 253 and Ser 254, and by one electrostatic interaction between Lys 154 (protein A) and Thr 256 (IgG). The second site is located in the heavy chain CH3 domain and is dominated by electrostatic interactions between Gln 129 and Tyr 133 (protein A) and His 433, Asn 434, and His 435 (IgG) (see Salvalaglio supra).

[0012] Lindhofer, H., et al. (J. Immunol. 155 (1995) 219-225) report preferential species-restricted heavy/light chain pairing in rat/mouse quadromas.

[0013] Jedenberg, L., et al. (J. Immunol. Meth. 201 (1997) 25-34) reported that SPA-binding analyses of two Fc variants (Fc13 and Fc31, each containing an isotypic dipeptide substitution from the respective other isotype) showed that Fc1 and Fc31 interact with SPA, while Fc3 and Fc13 lack detectable SPA binding. The rendered SPA binding of the Fc-region variant Fc31 is concluded to result from the introduced dipeptide substitution R435H and F436Y.

[0014] Today the focus with respect to therapeutic monoclonal antibodies is on the generation and use of bispecific or even multispecific antibodies specifically binding to two or more targets (antigens).

[0015] The basic challenge in generating multispecific heterodimeric IgG antibodies from four antibody chains (two different heavy chains and two different light chains) in one expression cell line is the so-called chain association issue (see Klein, C., et al., mAbs 4 (2012) 653-663). The required use of different chains as the left and the right arm

of the multispecific antibody leads to antibody mixtures upon expression in one cell: the two heavy chains are able to (theoretically) associate in four different combinations (two thereof are identical), and each of those can associate in a stochastic manner with the light chains, resulting in 2^4 (=a total of 16) theoretically possible chain combinations. Of the 16 theoretically possible combinations ten can be found of which only one corresponds to the desired functional bispecific antibody (De Lau, W. B., et al., J. Immunol. 146 (1991) 906-914). The difficulties in isolating this desired bispecific antibody out of complex mixtures and the inherent poor yield of 12.5% at a theoretical maximum make the production of a bispecific antibody in one expression cell line extremely challenging.

[0016] To overcome the chain association issue and enforce the correct association of the two different heavy chains, in the late 1990s Carter et al. from Genentech invented an approach termed "knobs-into-holes" (KiH) (see Carter, P., J. Immunol. Meth. 248 (2001) 7-15; Merchant, A. M., et al., Nat. Biotechnol. 16 (1998) 677-681; Zhu, Z., et al., Prot. Sci. 6 (1997) 781-788; Ridgway, J. B., et al., Prot. Eng. 9 (1996) 617-621; Atwell, S., et al., J. Mol. Biol. 270 (1997) 26-35; and U.S. Pat. No. 7,183,076). Basically, the concept relies on modifications of the interface between the two CH3 domains of the two heavy chains of an antibody where most interactions occur. A bulky residue is introduced into the CH3 domain of one antibody heavy chain and acts similarly to a key ("knob"). In the other heavy chain, a "hole" is formed that is able to accommodate this bulky residue, mimicking a lock. The resulting heterodimeric Fc-region can be further stabilized by the introduction/ formation of artificial disulfide bridges. Notably, all KiH mutations are buried within the CH3 domains and not "visible" to the immune system. In addition, properties of antibodies with KiH mutations such as (thermal) stability, FcyR binding and effector functions (e.g., ADCC, FcRn binding) and pharmacokinetic (PK) behavior are not affected.

[0017] Correct heavy chain association with heterodimerization yields above 97% can be achieved by introducing six mutations: S354C, T366W in the "knob" heavy chain and Y349C, T366S, L368A, Y407V in the "hole" heavy chain (see Carter supra; numbering of the residues according to the Kabat EU index numbering system). While hole-hole homodimers may occur, knob-knob homodimers typically are not observed. Hole-hole dimers can either be depleted by selective purification procedures or by procedures as outlined below.

[0018] While the issue of random heavy chain association has been addressed, also correct light chain association has to be ensured. Similar to the KiH CH3 domain approach, efforts have been undertaken to investigate asymmetric light chain-heavy chain interactions that might ultimately lead to full bispecific IgGs.

[0019] Roche recently developed the CrossMab approach as a possibility to enforce correct light chain pairing in bispecific heterodimeric IgG antibodies when combining it with the KiH technology (see Klein supra; Schaefer. W., et al., Proc. Natl. Acad. Sci. USA 108 (2011) 11187-11192; Cain, C., SciBX 4 (2011) 1-4). This allows the generation of bispecific or even multispecific antibodies in a generic fashion. In this format, one arm of the intended bispecific antibody is left untouched. In the second arm, the whole Fab region, or the VH-VL domains or the CH1-CL domains are

exchanged by domain crossover between the heavy and light chain. As a consequence, the newly formed "crossed" light chain does not associate with the (normal, i.e. not-crossed) heavy chain Fab region of the other arm of the bispecific antibody any longer. Thus, the correct "light chain" association can be enforced by this minimal change in domain arrangement (see Schaefer supra).

[0020] Zhu et al. introduced several sterically complementary mutations, as well as disulfide bridges, in the two VL/VH interfaces of diabody variants. When the mutations VL Y87A/F98M and VH V37F/L45W were introduced into the anti-p185HER2 VL/VH interface, a heterodimeric diabody was recovered with >90% yield while maintaining overall yield and affinity compared with the parental diabody (see Zhu supra).

[0021] Researchers from Chugai have similarly designed bispecific diabodies by introduction of mutations into the VH-VL interfaces (mainly conversion of Q39 in VH and Q38 in VL to charged residues) to foster correct light chain association (WO 2006/106905; Igawa, T., et al., Prot. Eng. Des. Sel. 23 (2010) 667-677).

[0022] In WO2011097603 a common light chain mouse is reported.

[0023] In WO2010151792 a bispecific antibody format providing ease of isolation is provided, comprising immunoglobulin heavy chain variable domains that are differentially modified, i.e. heterodimeric, in the CH3 domain, wherein the differential modifications are non-immunogenic or substantially non-immunogenic with respect to the CH3 modifications, and at least one of the modifications results in a differential affinity for the bispecific antibody for an affinity reagent such as protein A, and the bispecific antibody is isolable from a disrupted cell, from medium, or from a mixture of antibodies based on its affinity for protein A.

[0024] The neonatal Fc-receptor (FcRn) is important for the metabolic fate of antibodies of the IgG class in vivo. The FcRn functions to salvage IgG from the lysosomal degradation pathway, resulting in reduced clearance and increased half-life. It is a heterodimeric protein consisting of two polypeptides: a 50 kDa class I major histocompatibility complex-like protein (α -FcRn) and a 15 kDa β 2-microglobulin (β 2m). FcRn binds with high affinity to the CH2-CH3 portion of the Fc-region of an antibody of the class IgG. The interaction between an antibody of the class IgG and the FcRn is pH dependent and occurs in a 1:2 stoichiometry, i.e. one IgG antibody molecule can interact with two FcRn molecules via its two heavy chain Fc-region polypeptides (see e.g. Huber, A. H., et al., J. Mol. Biol. 230 (1993) 1077-1083).

[0025] Thus, an IgGs in vitro FcRn binding properties/ characteristics are indicative of its in vivo pharmacokinetic properties in the blood circulation.

[0026] In the interaction between the FcRn and the Fcregion of an antibody of the IgG class different amino acid residues of the heavy chain CH2- and CH3-domain are participating.

[0027] Different mutations that influence the FcRn binding and therewith the half-live in the blood circulation are known. Fc-region residues critical to the mouse Fc-regionmouse FcRn interaction have been identified by site-directed mutagenesis (see e.g. Dall'Acqua, W. F., et al. J. Immunol 169 (2002) 5171-5180). Residues I253, H310, H433, N434, and H435 (numbering according to Kabat EU index numbering system) are involved in the interaction (Medesan, C.,

et al., Eur. J. Immunol. 26 (1996) 2533-2536; Firan, M., et al., Int. Immunol. 13 (2001) 993-1002; Kim, J. K., et al., Eur. J. Immunol. 24 (1994) 542-548). Residues I253, H310, and H435 were found to be critical for the interaction of human Fc-region with murine FcRn (Kim, J. K., et al., Eur. J. Immunol. 29 (1999) 2819-2885).

[0028] Methods to increase Fc-region (and likewise IgG) binding to FcRn have been performed by mutating various amino acid residues in the Fc-region: Thr 250, Met 252, Ser 254, Thr 256, Thr 307, Glu 380, Met 428, His 433, and Asn 434 (see Kuo, T. T., et al., J. Clin. Immunol. 30 (2010) 777-789; Ropeenian, D. C., et al., Nat. Rev. Immunol. 7 (2007) 715-725).

[0029] The combination of the mutations M252Y, S254T, T256E have been described by Dall'Acqua et al. to improve FcRn binding by protein-protein interaction studies (Dall'Acqua, W. F., et al. J. Biol. Chem. 281 (2006) 23514-23524). Studies of the human Fc-region-human FcRn complex have shown that residues I253, S254, H435, and Y436 are crucial for the interaction (Firan, M., et al., Int. Immunol. 13 (2001) 993-1002; Shields, R. L., et al., J. Biol. Chem. 276 (2001) 6591-6604). In Yeung, Y. A., et al. (J. Immunol. 182 (2009) 7667-7671) various mutants of residues 248 to 259 and 301 to 317 and 376 to 382 and 424 to 437 have been reported and examined.

[0030] In WO 2014/006217 dimeric proteins with triple mutations are reported. Crystal structure at 2.8 Angstrom of an FcRn/heterodimeric Fc complex regarding the mechanism of pH-dependent binding was reported by Martin, W., et al. (Mol. Cell. 7 (2001) 867-877). In U.S. Pat. No. 6,277,375 immunoglobulin like domains with increased half-lives are reported in WO 2013/004842. Shields, R. L., et al., reported high resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R (Biochem. Mol. Biol. 276 (2001) 6591-6604). The delineation of the amino acid residues involved in transcytosis and catabolism of mouse IgG1 was reported by Medesan, C., et al. (J. Immunol. 158 (1997) 2211-2217). In US 2010/0272720 antibody fusion proteins with a modified FcRn binding site are reported. The production of heterodimeric proteins is reported in WO 2013/ 060867. Oiao, S.-W., et al. reported the dependence of antibody-mediated presentation of antigen on FcRn (Proc. Natl. Acad. Sci. USA 105 (2008) 9337-9342.

SUMMARY OF THE INVENTION

[0031] Herein are reported variant Fc-regions that specifically bind to *Staphylococcus* protein A and that do not bind to human FcRn. These variant Fc-regions contain specific amino acid mutations in the CH2- and CH3-domain. It has been found that these mutations when used either in the hole chain or the knob chain of a heterodimeric Fc-region allow for the purification of the heterodimeric Fc-region, i.e. the separation of a heterodimeric Fc-region from a homodimeric Fc-region.

[0032] One aspect as reported herein is a (dimeric) polypeptide comprising

[0033] a first polypeptide comprising in N-terminal to C-terminal direction at least a portion of an immunoglobulin hinge region, which comprises one or more cysteine residues, an immunoglobulin CH2-domain and an immunoglobulin CH3-domain, and a second polypeptide comprising in N-terminal to C-terminal

direction at least a portion of an immunoglobulin hinge region, which comprises one or more cysteine residues, an immunoglobulin CH2-domain and an immunoglobulin CH3-domain,

[0034] wherein (numbering according to the Kabat EU index numbering system)

[0035] i) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or

[0036] ii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or

[0037] iii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S

[0038] and,

[0039] wherein the first polypeptide and the second polypeptide are connected by one or more disulfide bridges in the at least a portion of an immunoglobulin hinge region.

[0040] In one embodiment the (dimeric) polypeptide does not specifically bind to the human FcRn and does specifically bind to Staphylococcal protein A.

[0041] In one embodiment the (dimeric) polypeptide is a homodimeric polypeptide.

[0042] In one embodiment the (dimeric) polypeptide is a heterodimeric polypeptide.

[0043] In one embodiment the first polypeptide further comprises the mutations Y349C, T366S, L368A and Y407V ("hole") and the second polypeptide comprises the mutations S354C and T366W ("knob").

[0044] In one embodiment the first polypeptide further comprises the mutations S354C, T366S, L368A and Y407V ("hole") and the second polypeptide comprises the mutations Y349C and T366W ("knob").

[0045] In one embodiment the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain of the first and the second polypeptide are of the human IgG1 subclass. In one embodiment the first polypeptide and the second polypeptide each further comprise the mutations L234A and L235A. In one embodiment the first polypeptide and the second polypeptide each further comprise the mutation P329G. In one embodiment the first polypeptide and the second polypeptide each further comprise the mutations L234A, L235A and P329G.

[0046] In one embodiment the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain of the first and the second polypeptide are of the human IgG2 subclass. In one embodiment the first polypeptide and the second polypeptide each further comprise the mutations H268Q, V309L, A330S and P331S.

[0047] In one embodiment the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain of the first and the second polypeptide are of the human IgG2 subclass. In one embodiment the first polypeptide and the second polypeptide each further comprise the mutations V234A, G237A, P238S, H268A, V309L, A330S and P331S.

[0048] In one embodiment the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain of the first and the second polypeptide

are of the human IgG4 subclass. In one embodiment the first polypeptide and the second polypeptide each further comprise the mutations S228P and L235E. In one embodiment the first polypeptide and the second polypeptide each further comprise the mutation P329G. In one embodiment the first polypeptide and the second polypeptide each further comprise the mutations S228P, L235E and P329G.

[0049] In one embodiment the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain of the first and the second polypeptide are of the human IgG4 subclass. In one embodiment the first polypeptide and the second polypeptide each further comprise the mutations S228P, L234A and L235A. In one embodiment the first polypeptide and the second polypeptide each further comprise the mutation P329G. In one embodiment the first polypeptide and the second polypeptide each further comprise the mutations S228P, L234A, L235A and P329G.

[0050] In one embodiment the first and the second polypeptide further comprise the mutation Y436A.

[0051] In one embodiment the (dimeric) polypeptide is an Fc-region fusion polypeptide.

[0052] In one embodiment the (dimeric) polypeptide is an (full length) antibody.

[0053] In one embodiment the (full length) antibody is a monospecific antibody. In one embodiment the monospecific antibody is a monovalent monospecific antibody. In one embodiment the monospecific antibody is a bivalent monospecific antibody.

[0054] In one embodiment the (full length) antibody is a bispecific antibody. In one embodiment the bispecific antibody is a bivalent bispecific antibody. In one embodiment the bispecific antibody is a tetravalent bispecific antibody.

[0055] In one embodiment the (full length) antibody is a trispecific antibody. In one embodiment the trispecific antibody is a trivalent trispecific antibody. In one embodiment the trispecific antibody is a tetravalent trispecific antibody.

[0056] One aspect as reported herein is an antibody comprising

- [0057] a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1 and an immunoglobulin CH3-domain of the subclass IgG1,
- [0058] a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1 and an immunoglobulin CH3-domain of the subclass IgG1.
- [0059] a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and a light chain constant domain,
- [0060] a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,
- [0061] wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen,

- [0062] wherein the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a second antigen,
- [0063] wherein i) the first polypeptide comprises the mutations Y349C, T366S, L368A, and Y407V, L234A, L235A and P329G and the second polypeptide comprises the mutations S354C, and T366W, L234A, L235A and P329G, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A, Y407V, L234A, L235A and P329G and the second polypeptide comprises the mutations Y349C, T366W, L234A, L235A and P329G, and
- [0064] wherein (numbering according to the Kabat EU index numbering system)
 - [0065] i) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
 - [0066] ii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
 - [0067] iii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S,

[0068] and

[0069] wherein the first polypeptide and the second polypeptide are connected by one or more disulfide bridges in the hinge region.

[0070] One aspect as reported herein is an antibody comprising

- [0071] a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin light chain constant domain, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1 and an immunoglobulin CH3-domain of the subclass IgG1.
- [0072] a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1 and an immunoglobulin CH3-domain of the subclass IgG1,
- [0073] a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and an immunoglobulin CH1-domain of the subclass IgG1,
- [0074] a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,
- [0075] wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen,
- [0076] wherein the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a second antigen, wherein i) the first polypeptide comprises the mutations Y349C, T366S, L368A, and Y407V, L234A, L235A and P329G and the second polypeptide comprises the mutations S354C, and T366W, L234A,

- L235A and P329G, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A, Y407V, L234A, L235A and P329G and the second polypeptide comprises the mutations Y349C, T366W, L234A, L235A and P329G, and
- [0077] wherein (numbering according to the Kabat EU index numbering system)
 - [0078] i) the first polypeptide comprises the mutations 1253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
 - [0079] ii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
 - [0080] iii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S,

[0081] and

- [0082] wherein the first polypeptide and the second polypeptide are connected by one or more disulfide bridges in the hinge region.
- [0083] One aspect as reported herein is an antibody comprising
 - [0084] a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG4, an immunoglobulin hinge region of the subclass IgG4 and an immunoglobulin CH2-domain of the subclass IgG4,
 - [0085] a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG4, an immunoglobulin hinge region of the subclass IgG4, an immunoglobulin CH2-domain of the subclass IgG4 and an immunoglobulin CH3-domain of the subclass IgG4,
 - [0086] a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and a light chain constant domain.
 - [0087] a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,
 - [0088] wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen,
 - [0089] wherein the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a second antigen,
 - [0090] wherein i) the first polypeptide comprises the mutations Y349C, T366S, L368A, and Y407V, S228P, L235E and P329G and the second polypeptide comprises the mutations S354C, and T366W, S228P, L235E and P329G, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A, Y407V, S228P, L235E and P329G and the second polypeptide comprises the mutations Y349C, T366W, S228P, L235E and P329G, and
 - [0091] wherein (numbering according to the Kabat EU index numbering system)

- [0092] i) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
- [0093] ii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
- [0094] iii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S,

[0095] and

- [0096] wherein the first polypeptide and the second polypeptide are connected by one or more disulfide bridges in the hinge region.
- [0097] One aspect as reported herein is an antibody comprising
 - [0098] a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin light chain constant domain, an immunoglobulin hinge region of the subclass IgG4, an immunoglobulin CH2-domain of the subclass IgG4 and an immunoglobulin CH3-domain of the subclass IgG4,
 - [0099] a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG4, an immunoglobulin hinge region of the subclass IgG4, an immunoglobulin CH2-domain of the subclass IgG4 and an immunoglobulin CH3-domain of the subclass IgG4.
 - [0100] a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and an immunoglobulin CH1-domain of the subclass IgG4,
 - [0101] a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,
 - [0102] wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen,
 - [0103] wherein the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a second antigen,
 - [0104] wherein i) the first polypeptide comprises the mutations Y349C, T366S, L368A, and Y407V, S228P, L235E and P329G and the second polypeptide comprises the mutations S354C, and T366W, S228P, L235E and P329G, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A, Y407V, S228P, L235E and P329G and the second polypeptide comprises the mutations Y349C, T366W, S228P, L235E and P329G, and
 - [0105] wherein (numbering according to the Kabat EU index numbering system)
 - [0106] i) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or

- [0107] ii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
- [0108] iii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S,

[0109] and

- [0110] wherein the first polypeptide and the second polypeptide are connected by one or more disulfide bridges in the hinge region.
- [0111] One aspect as reported herein is an antibody comprising
 - [0112] a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1, an immunoglobulin CH3-domain of the subclass IgG1, a peptidic linker and a first scFv,
 - [0113] a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1, an immunoglobulin CH3-domain of the subclass IgG1, a peptidic linker and a second scFv,
 - [0114] a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and a light chain constant domain,
 - [0115] a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,
 - [0116] wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen, and the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a first antigen, and the first and the second scFv specifically bind to a second antigen,
 - [0117] wherein i) the first polypeptide comprises the mutations Y349C, T366S, L368A, and Y407V, L234A, L235A and P329G and the second polypeptide comprises the mutations S354C, and T366W, L234A, L235A and P329G, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A, Y407V, L234A, L235A and P329G and the second polypeptide comprises the mutations Y349C, T366W, L234A, L235A and P329G, and
 - [0118] wherein (numbering according to the Kabat EU index numbering system)
 - [0119] i) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
 - [0120] ii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
 - [0121] iii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S,

- [0122] and
- [0123] wherein the first polypeptide and the second polypeptide are connected by one or more disulfide bridges in the hinge region.
- [0124] One aspect as reported herein is an antibody comprising
 - [0125] a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin light chain constant domain, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1, an immunoglobulin CH3-domain of the subclass IgG1, a peptidic linker and a first scFv,
 - [0126] a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1, an immunoglobulin CH3-domain of the subclass IgG1, a peptidic linker and a second scFv,
 - [0127] a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and an immunoglobulin CH1-domain of the subclass IgG1,
 - [0128] a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,
 - [0129] wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen, and the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a first antigen, and the first and the second scFv specifically bind to a second antigen,
 - [0130] wherein i) the first polypeptide comprises the mutations Y349C, T366S, L368A, and Y407V, L234A, L235A and P329G and the second polypeptide comprises the mutations S354C, and T366W, L234A, L235A and P329G, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A, Y407V, L234A, L235A and P329G and the second polypeptide comprises the mutations Y349C, T366W, L234A, L235A and P329G, and
 - [0131] wherein (numbering according to the Kabat EU index numbering system)
 - [0132] i) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
 - [0133] ii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
 - [0134] iii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S,

[0135] and

- [0136] wherein the first polypeptide and the second polypeptide are connected by one or more disulfide bridges in the hinge region.
- [0137] One aspect as reported herein is a method for producing a (dimeric) polypeptide as reported herein comprising the following steps:

- [0138] a) cultivating a mammalian cell comprising one or more nucleic acids encoding the (dimeric) polypeptide.
- [0139] b) recovering the (dimeric) polypeptide from the cultivation medium, and
- [0140] c) purifying the (dimeric) polypeptide with a protein A affinity chromatography and thereby producing the (dimeric) polypeptide.
- [0141] One aspect as reported herein is the use of the combination of the mutations H310A, H433A and Y436A for separating heterodimeric polypeptides from homodimeric polypeptides.
- **[0142]** One aspect as reported herein is the use of the combination of the mutations L251D, L314D and L432D for separating heterodimeric polypeptides from homodimeric polypeptides.
- [0143] One aspect as reported herein is the use of the combination of the mutations L251S, L314S and L432S for separating heterodimeric polypeptides from homodimeric polypeptides.
- [0144] One aspect as reported herein is the use of the combination of the mutations I253A, H310A and H435A in a first Fc-region polypeptide in combination with the combination of the mutations H310A, H433A and Y436A in a second Fc-region polypeptide for separating heterodimeric Fc-regions comprising the first and the second Fc-region polypeptide from homodimeric Fc-regions.
- [0145] One aspect as reported herein is the use of the combination of the mutations I253A, H310A and H435A in a first Fc-region polypeptide in combination with the combination of the mutations L251D, L314D and L432D in a second Fc-region polypeptide for separating heterodimeric Fc-regions comprising the first and the second Fc-region polypeptide from homodimeric Fc-regions.
- [0146] One aspect as reported herein is the use of the combination of the mutations I253A, H310A and H435A in a first Fc-region polypeptide in combination with the combination of the mutations L251S, L314S and L432S in a second Fc-region polypeptide for separating heterodimeric Fc-regions comprising the first and the second Fc-region polypeptide from homodimeric Fc-regions.
- [0147] In one embodiment of the previous three aspects i) the first Fc-region polypeptide further comprises the mutations Y349C, T366S, L368A and Y407V and the second Fc-region polypeptide further comprises the mutations S354C and T366W, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A, and Y407V, and the second polypeptide comprises the mutations Y349C and T366W.
- [0148] In one embodiment both Fc-region polypeptides comprise in N-terminal to C-terminal direction a heavy chain variable domain, an immunoglobulin CH1-domain, an immunoglobulin hinge region, an immunoglobulin CH2-domain and an immunoglobulin CH3-domain. In one embodiment the hinge region and the immunoglobulin domains are all of the IgG1 subclass or all of the IgG4 subclass.
- [0149] One aspect as reported herein is method of treatment of a patient suffering from ocular vascular diseases by administering a (dimeric) polypeptide or antibody as reported herein to a patient in the need of such treatment.
- [0150] One aspect as reported herein is a (dimeric) polypeptide or an antibody as reported herein for intravitreal application.

- [0151] One aspect as reported herein is a (dimeric) polypeptide or an antibody as reported herein for use as a medicament.
- [0152] One aspect as reported herein is a (dimeric) polypeptide or an antibody as reported herein for the treatment of vascular eye diseases.
- [0153] One aspect as reported herein is a pharmaceutical formulation comprising a (dimeric) polypeptide or an antibody as reported herein and optionally a pharmaceutically acceptable carrier.
- [0154] For using an antibody that targets/binds to antigens not only present in the eye but also in the remaining body a short systemic half-live after passage of the blood-ocular-barrier from the eye into the blood is beneficial in order to avoid systemic side effects.
- [0155] Additionally an antibody that specifically binds to ligands of a receptor is only effective in the treatment of eye-diseases if the antibody-antigen complex is removed from the eye, i.e. the antibody functions as a transport vehicle for receptor ligands out of the eye and thereby inhibits receptor signaling.
- [0156] It has been found by the current inventors that an antibody comprising an Fc-region that does not bind to the human neonatal Fc-receptor, i.e. a (dimeric) polypeptide as reported herein, is transported across the blood-ocular barrier. This is surprising as the antibody does not bind to human FcRn although binding to FcRn is considered to be required for transport across the blood-ocular-barrier.
- [0157] One aspect as reported herein is the use of a (dimeric) polypeptide or an antibody as reported herein for the transport of a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.
- [0158] One aspect as reported herein is the use of a (dimeric) polypeptide or an antibody as reported herein for the removal of one or more soluble receptor ligands from the eye.
- [0159] One aspect as reported herein is the use of a (dimeric) polypeptide or an antibody as reported herein for the treatment of eye diseases, especially of ocular vascular diseases.
- **[0160]** One aspect as reported herein is the use of a (dimeric) polypeptide or an antibody as reported herein for the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.
- [0161] One aspect as reported herein is a (dimeric) polypeptide or an antibody as reported herein for use in treating an eye disease.
- [0162] One aspect as reported herein is a (dimeric) polypeptide or an antibody as reported herein for use in the transport of a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.
- [0163] One aspect as reported herein is a (dimeric) polypeptide or an antibody as reported herein for use in the removal of one or more soluble receptor ligands from the eye.
- [0164] One aspect as reported herein is a (dimeric) polypeptide or an antibody as reported herein for use in treating eye diseases, especially ocular vascular diseases.
- [0165] One aspect as reported herein is a (dimeric) polypeptide or an antibody as reported herein for use in the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.
- [0166] One aspect as reported herein is a method of treating an individual having an ocular vascular disease

comprising administering to the individual an effective amount of a (dimeric) polypeptide or an antibody as reported herein.

[0167] One aspect as reported herein is a method for transporting a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation in an individual comprising administering to the individual an effective amount of a (dimeric) polypeptide or an antibody as reported herein to transport a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation

[0168] One aspect as reported herein is a method the removal of one or more soluble receptor ligands from the eye in an individual comprising administering to the individual an effective amount of a (dimeric) polypeptide or an antibody as reported herein to remove one or more soluble receptor ligands from the eye.

[0169] One aspect as reported herein is a method for the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation in an individual comprising administering to the individual an effective amount of a (dimeric) polypeptide or an antibody as reported herein to transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.

[0170] One aspect as reported herein is a method for transporting a soluble receptor ligand from the intravitreal space or the eye over the blood-ocular-barrier into the blood circulation in an individual comprising administering to the individual an effective amount of a (dimeric) polypeptide or an antibody as reported herein to transport a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.

[0171] In one embodiment the (dimeric) polypeptide is a bispecific antibody. In one embodiment the bispecific antibody is a bivalent bispecific antibody. In one embodiment the bispecific antibody is a tetravalent bispecific antibody.

[0172] In one embodiment the (dimeric) polypeptide is a trispecific antibody. In one embodiment the trispecific antibody is a trivalent trispecific antibody. In one embodiment the trispecific antibody is a tetravalent trispecific antibody.

[0173] In one embodiment the (dimeric) polypeptide is a

[0173] In one embodiment the (dimeric) polypeptide is a CrossMab.

[0174] In one embodiment the (dimeric) polypeptide is an Fc-region fusion polypeptide.

[0175] In one embodiment the first polypeptide further comprises the mutations Y349C, T366S, L368A and Y407V and the second polypeptide further comprises the mutations S354C and T366W.

[0176] In one embodiment the first polypeptide further comprises the mutations S354C, T366S, L368A and Y407V and the second polypeptide further comprises the mutations Y349C and T366W.

[0177] In one embodiment the antibody or the Fc-region fusion polypeptide is of the subclass IgG1. In one embodiment the antibody or the Fc-region fusion polypeptide further comprise the mutations L234A and L235A. In one embodiment the antibody or the Fc-region fusion polypeptide further comprise the mutation P329G.

[0178] In one embodiment the antibody or the Fc-region fusion polypeptide is of the subclass IgG2. In one embodiment the antibody or the Fc-region fusion polypeptide further comprise the mutations V234A, G237A, P238S, H268A, V309L, A330S and P331S.

[0179] In one embodiment the antibody or the Fc-region fusion polypeptide is of the subclass IgG4. In one embodiment the antibody or the Fc-region fusion polypeptide further comprise the mutations S228P and L235E. In one embodiment the antibody or the Fc-region fusion polypeptide further comprise the mutation P329G.

BRIEF DESCRIPTION OF THE FIGURES

[0180] FIG. 1: Scheme of concept and advantages of anti-VEGF/ANG2 antibodies of the IgG1 or IgG4 subclass with IHH-AAA mutation (combination of mutations I253A, H310A and H435A (numbering according to the Kabat EU index numbering system)).

[0181] FIG. 2: Small-scale DLS-based viscosity measurement: Extrapolated viscosity at 150 mg/mL in 200 mM arginine/succinate buffer, pH 5.5 (comparison of anti-VEGF/ANG2 antibody VEGF/ANG2-0016 (with IHH-AAA mutation) with reference antibody VEGF/ANG2-0015 (without such IHH-AAA mutation)).

[0182] FIG. 3: DLS Aggregation depending on temperature (including DLS aggregation onset temperature) in 20 mM histidine buffer, 140 mM NaCl, pH 6.0 (comparison of anti-VEGF/ANG2 antibody as reported herein VEGF/ANG2-0016 (with IHH-AAA mutation) with reference antibody VEGF/ANG2-0015 (without such IHH-AAA mutation)).

[0183] FIG. 4: Seven day storage at 40° C. at 100 mg/mL (decrease of Main Peak and High Molecular Weight (HMW) increase) (comparison of anti-VEGF/ANG2 antibody as reported herein VEGF/ANG2-0016 (with IHH-AAA mutation) which showed a lower aggregation with reference antibody VEGF/ANG2-0015 (without such IHH-AAA mutation)).

[0184] FIGS. 5A and 5B: FcRn steady state affinity of A: VEGF/ANG2-0015 (without IHH-AAA mutation) and B: VEGF/ANG2-0016 (with IHH-AAA mutation).

[0185] FIG. 6: FcgammaRIIIa interaction measurement of VEGF/ANG2-0015 without IHH-AAA mutation and VEGF/ANG2-0016 with IHH-AAA mutation (both are IgG1 subclass with P329G LALA mutations; as controls an anti-digoxygenin antibody (anti-Dig antibody) of IgG1 subclass and an IgG4 based antibody were used).

[0186] FIG. 7A: Schematic pharmacokinetic (PK) ELISA assay principle for determination of concentrations of anti-VEGF/ANG2 antibodies in serum and whole eye lysates.

[0187] FIG. 7B: Serum concentration after intravenous (i.v.) application: comparison of VEGF/ANG2-0015 without IHH-AAA mutation and VEGF/ANG2-0016 with IHH-AAA mutation.

[0188] FIG. 7C: Serum concentration after intravitreal application: comparison of VEGF/ANG2-0015 without IHH-AAA mutation and VEGF/ANG2-0016 with IHH-AAA mutation.

[0189] FIG. 7D: Eye lysates concentration of VEGF/ANG2-0016 (with IHH-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 concentration in eye lysates could be detected due to the low serum half-life of VEGF/ANG2-0016 (with IHH-AAA mutation).

[0190] FIG. 7E: Eye lysates concentration of VEGF/ANG2-0015 (without IHH-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 VEGF/ANG2-0015 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 VEGF/ANG2-0015 (without IHH-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 VEGF/ANG2-0015 (without IHH-AAA mutation).

[0191] FIGS. 8A, 8B and 8C: Antibodies engineered with respect to their ability to bind FcRn display prolonged (YTE mutation) or shortened (IHH-AAA mutation) in vivo half-lives, enhanced (YTE mutation) or reduced binding (IHH-AAA mutation) compared to the reference wild-type (wt) antibody in SPR analysis as well as enhanced or reduced retention time in FcRn column chromatography; 8A PK data after single i.v. bolus application of 10 mg/kg into huFcRn transgenic male C57BL/6J mice +/-276: AUC data for wt IgG as well as YTE and IHH-AAA Fc-region-modified IgGs; 8B BIAcore sensorgram; 8C FcRn affinity column elution; wild-type anti-IGF-1R antibody (reference), YTE-mutant of anti-IGF-1R antibody.

[0192] FIG. 9: Change of retention time in an FcRn affinity chromatography depending on the number of mutations introduced into the Fc-region.

[0193] FIG. 10: Change of FcRn-binding depending on asymmetric distribution of mutations introduced into the Fc-region.

[0194] FIG. 11: Elution chromatogram of a bispecific anti-VEGF/ANG2 antibody (VEGF/ANG2-0121) with the combination of the mutations H310A, H433A and Y436A in both heavy chains from two consecutive protein A affinity chromatography columns.

[0195] FIG. 12: Elution chromatogram of an anti-IGF-1R antibody (IGF-1R-0045) with the mutations H310A, H433A and Y436A in both heavy chains from a protein A affinity chromatography column.

[0196] FIG. 13: Binding of IgG Fc-region modified anti-VEGF/ANG2 antibodies to immobilized protein A on a CM5 chip.

[0197] FIG. 14: Elution chromatogram of different anti-VEGF/ANG2 antibodies on an FcRn affinity column.

[0198] FIG. 15: Binding of different fusion polypeptides to Staphylococcal protein A (SPR).

[0199] FIG. 16: Binding of different anti-VEGF/ANG2 antibody and anti-IGF-1R antibody mutants to immobilized protein A (SPR).

[0200] FIG. 17: Comparison of serum concentrations after intravenous application of antibodies IGF-1R 0033, 0035 and 0045.

[0201] FIG. 18: Comparison of eye lysate concentration after intravitreal and intravenous application of antibody IGF-1R 0033.

[0202] FIG. 19: Comparison of eye lysate concentration after intravitreal and intravenous application of antibody IGF-1R 0035.

[0203] FIG. 20: Comparison of eye lysate concentration after intravitreal and intravenous application of antibody IGF-1R 0045.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

I. Definitions

[0204] The term "about" denotes a range of $\pm -20\%$ of the thereafter following numerical value. In one embodiment the term about denotes a range of $\pm -10\%$ of the thereafter following numerical value. In one embodiment the term about denotes a range of $\pm -5\%$ of the thereafter following numerical value.

[0205] An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence alterations. In some embodiments, the number of amino acid alterations are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[0206] An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the inity of the antibody for antigen.

[0207] The term "alteration" denotes the mutation (substitution), insertion (addition), or deletion of one or more amino acid residues in a parent antibody or fusion polypeptide, e.g. a fusion polypeptide comprising at least an FcRn binding portion of an Fc-region, to obtain a modified antibody or fusion polypeptide. The term "mutation" denotes that the specified amino acid residue is substituted for a different amino acid residue. For example the mutation L234A denotes that the amino acid residue lysine at position 234 in an antibody Fc-region (polypeptide) is substituted by the amino acid residue alanine (substitution of lysine with alanine) (numbering according to the Kabat EU index numbering system).

[0208] A "naturally occurring amino acid residues" denotes an amino acid residue from the group consisting of alanine (three letter code: Ala, one letter code: A), arginine (Arg, R), asparagine (Asn, N), aspartic acid (Asp, D), cysteine (Cys, C), glutamine (Gln, Q), glutamic acid (Glu, E), glycine (Gly, G), histidine (His, H), isoleucine (Ile, I), leucine (Leu, L), lysine (Lys, K), methionine (Met, M), phenylalanine (Phe, F), proline (Pro, P), serine (Ser, S), threonine (Thr, T), tryptophane (Trp, W), tyrosine (Tyr, Y), and valine (Val, V).

[0209] The term "amino acid mutation" denotes the substitution of at least one existing amino acid residue with another different amino acid residue (=replacing amino acid residue). The replacing amino acid residue may be a "naturally occurring amino acid residues" and selected from the group consisting of alanine (three letter code: ala, one letter code: A), arginine (arg, R), asparagine (asn, N), aspartic acid (asp, D), cysteine (cys, C), glutamine (gln, Q), glutamic acid (glu, E), glycine (gly, G), histidine (his, H), isoleucine (ile,

I), leucine (leu, L), lysine (lys, K), methionine (met, M), phenylalanine (phe, F), proline (pro, P), serine (ser, S), threonine (thr, T), tryptophan (trp, W), tyrosine (tyr, Y), and valine (val, V). The replacing amino acid residue may be a "non-naturally occurring amino acid residue". See e.g. U.S. Pat. No. 6,586,207, WO 98/48032, WO 03/073238, US 2004/0214988, WO 2005/35727, WO 2005/74524, Chin, J. W., et al., J. Am. Chem. Soc. 124 (2002) 9026-9027; Chin, J. W. and Schultz, P. G., ChemBioChem 11 (2002) 1135-1137; Chin, J. W., et al., PICAS United States of America 99 (2002) 11020-11024; and, Wang, L. and Schultz, P. G., Chem. (2002) 1-10 (all entirely incorporated by reference herein).

[0210] The term "amino acid deletion" denotes the removal of at least one amino acid residue at a predetermined position in an amino acid sequence.

[0211] The term "ANG-2" as used herein refers to human angiopoietin-2 (ANG-2) (alternatively abbreviated with ANGPT2 or ANG2) (SEQ ID NO: 31) 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: 32) 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) 242-248). 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) 353-356; Kim, I., et al., J. Biol. Chem. 274 (1999) 26523-26528). 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) 1161-1169; 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: 33), 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).

[0212] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, multispecific antibodies (e.g. bispecific antibodies, trispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-, and/or protein A and/or FcRn-binding activity.

[0213] The term "asymmetric Fc-region" denotes a pair of Fc-region polypeptides that have different amino acid residues at corresponding positions according to the Kabat EU index numbering system.

[0214] The term "asymmetric Fc-region with respect to FcRn binding" denotes an Fc-region that consists of two polypeptide chains that have different amino acid residues at corresponding positions, whereby the positions are determined according to the Kabat EU index numbering system, whereby the different positions affect the binding of the Fc-region to the human neonatal Fc-receptor (FcRn). For the purpose herein the differences between the two polypeptide chains of the Fc-region in an "asymmetric Fc-region with respect to FcRn binding" do not include differences that have been introduced to facilitate the formation of heterodimeric Fc-regions, e.g. for the production of bispecific antibodies. These differences can also be asymmetric, i.e. the two chains have differences at non corresponding amino acid residues according to the Kabat EU index numbering system. These differences facilitate heterodimerization and reduce homodimerization. Examples of such differences are the so-called "knobs into holes" substitutions (see, e.g., U.S. Pat. No. 7,695,936 and US 2003/0078385). The following knobs and holes substitutions in the individual polypeptide chains of an Fc-region of an IgG antibody of subclass IgG1 have been found to increase heterodimer formation: 1) Y407T in one chain and T366Y in the other chain; 2) Y407A in one chain and T366W in the other chain; 3) F405A in one chain and T394W in the other chain; 4) F405W in one chain and T394S in the other chain; 5) Y407T in one chain and T366Y in the other chain; 6) T366Y and F405A in one chain and T394W and Y407T in the other chain; 7) T366W and F405W in one chain and T394S and Y407A in the other chain; 8) F405W and Y407A in one chain and T366W and T394S in the other chain; and 9) T366W in one chain and T366 S, L368A, and Y407V in the other chain, whereby the last listed is especially suited. In addition, changes creating new disulfide bridges between the two Fc-region polypeptide chains facilitate heterodimer formation (see, e.g., US 2003/0078385). The following substitutions resulting in appropriately spaced apart cysteine residues for the formation of new intra-chain disulfide bonds in the individual polypeptide chains of an Fc-region of an IgG antibody of subclass IgG1 have been found to increase heterodimer formation: Y349C in one chain and S354C in the other; Y349C in one chain and E356C in the other; Y349C in one chain and E357C in the other; L351C in one chain and S354C in the other; T394C in one chain and E397C in the other; or D399C in one chain and K392C in the other. Further examples of heterodimerization facilitating amino acid changes are the so-called "charge pair substitutions" (see, e.g., WO 2009/089004). The following charge pair substitutions in the individual polypeptide chains of an Fc-region of an IgG antibody of subclass IgG1 have been found to increase heterodimer formation: 1) K409D or K409E in one chain and D399K or D399R in the other chain; 2) K392D or K392E in one chain and D399K or D399R in the other chain; 3) K439D or K439E in one chain and E356K or E356R in the other chain; 4) K370D or K370E in one chain and E357K or E357R in the other chain; 5) K409D and K360D in one chain plus D399K and E356K in the other chain; 6) K409D and K370D in one chain plus D399K and E357K in the other chain; 7) K409D and K392D in one chain plus D399K, E356K, and E357K in the other chain; 8) K409D and K392D in one chain and D399K in the other chain; 9) K409D and K392D in one chain and D399K and E356K in the other chain; 10) K409D and K392D in one chain and D399K and D357K in the other chain; 11) K409D and K370D in one chain and D399K and D357K in the other chain; 12) D399K in one chain and K409D and K360D in the other chain; and 13) K409D and K439D in one chain and D399K and E356K on the other.

[0215] The term "binding (to an antigen)" denotes the binding of an antibody to its antigen in an in vitro assay, in one embodiment in a binding assay in which the antibody is bound to a surface and binding of the antigen to the antibody is measured by Surface Plasmon Resonance (SPR). Binding means a binding affinity (K_D) of 10^{-8} M or less, in some embodiments of 10^{-13} to 10^{-8} M, in some embodiments of 10^{-13} to 10^{-9} M.

[0216] Binding can be investigated by a BIAcore assay (GE Healthcare Biosensor AB, Uppsala, Sweden). The affinity of the binding is defined by the terms k_a (rate constant for

the association of the antibody from the antibody/antigen complex), \mathbf{k}_d (dissociation constant), and $\mathbf{K}_D(\mathbf{k}_d/\mathbf{k}_a)$.

[0217] The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0218] The term "CH2-domain" denotes the part of an antibody heavy chain polypeptide that extends approximately from EU position 231 to EU position 340 (EU numbering system according to Kabat). In one embodiment a CH2 domain has the amino acid sequence of SEQ ID NO: 09: APELLGG PSVFLFPPKP KDTLMISRTP EVTCVWDVS HEDPEVKFNW YVDGVEVHNA KTK-PREEQ E STYRWSVLT VLHQDWLNGK EYKCK-VSNKA LPAPIEKTIS KAK.

[0219] The term "CH3-domain" denotes the part of an antibody heavy chain polypeptide that extends approximately from EU position 341 to EU position 446. In one embodiment the CH3 domain has the amino acid sequence of SEQ ID NO: 10: GQPREPQ VYTLPPSRDE LTKNQVS-LTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG.

[0220] The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively.

[0221] The term "comparable length" denotes that two polypeptides comprise the identical number of amino acid residues or can be different in length by one or more and up to 10 amino acid residues at most. In one embodiment the (Fc-region) polypeptides comprise the identical number of amino acid residues or differ by a number of from 1 to 10 amino acid residues. In one embodiment the (Fc-region) polypeptides comprise the identical number of amino acid residues or differ by a number of from 1 to 5 amino acid residues. In one embodiment the (Fc-region) polypeptides comprise the identical number of amino acid residues or differ by a number of from 1 to 3 amino acid residues.

[0222] "Effector functions" refer to those biological activities attributable to the Fc-region of an antibody, which vary with the antibody class. Examples of antibody effector functions include: Clq binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B-cell activation.

[0223] An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0224] The term "Fc-fusion polypeptide" denotes a fusion of a binding domain (e.g. an antigen binding domain such as a single chain antibody, or a polypeptide such as a ligand of a receptor) with an antibody Fc-region that exhibits the desired target-, protein A- and FcRn-binding activity.

[0225] The term "Fc-region of human origin" denotes the C-terminal region of an immunoglobulin heavy chain of human origin that contains at least a part of the hinge region,

the CH2 domain and the CH3 domain. In one embodiment, a human IgG heavy chain Fc-region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. In one embodiment the Fc-region has the amino acid sequence of SEQ ID NO: 60. However, the C-terminal lysine (Lys447) of the Fc-region may or may not be present.

[0226] As used herein, the amino acid positions of all constant regions and domains of the heavy and light chain are numbered according to the Kabat numbering system described in Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, Md. (1991) and is referred to as "numbering according to Kabat" herein. Specifically the Kabat numbering system (see pages 647-660) of Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, Md. (1991) is used for the light chain constant domain CL of kappa and lambda isotype and the Kabat EU index numbering system (see pages 661-723) is used for the constant heavy chain domains (CH1, Hinge, CH2 and CH3).

[0227] The term "FcRn" denotes the human neonatal Fc-receptor. FcRn functions to salvage IgG from the lysosomal degradation pathway, resulting in reduced clearance and increased half-life. The FcRn is a heterodimeric protein consisting of two polypeptides: a 50 kDa class I major histocompatibility complex-like protein (α-FcRn) and a 15 kDa β 2-microglobulin (β 2m). FcRn binds with high affinity to the CH2-CH3 portion of the Fc-region of IgG. The interaction between IgG and FcRn is strictly pH dependent and occurs in a 1:2 stoichiometry, with one IgG binding to two FcRn molecules via its two heavy chains (Huber, A. H., et al., J. Mol. Biol. 230 (1993) 1077-1083). FcRn binding occurs in the endosome at acidic pH (pH<6.5) and IgG is released at the neutral cell surface (pH of about 7.4). The pH-sensitive nature of the interaction facilitates the FcRnmediated protection of IgGs pinocytosed into cells from intracellular degradation by binding to the receptor within the acidic environment of endosomes. FcRn then facilitates the recycling of IgG to the cell surface and subsequent release into the blood stream upon exposure of the FcRn-IgG complex to the neutral pH environment outside the cell.

[0228] The term "FcRn binding portion of an Fc-region" denotes the part of an antibody heavy chain polypeptide that extends approximately from EU position 243 to EU position 261 and approximately from EU position 275 to EU position 293 and approximately from EU position 302 to EU position 319 and approximately from EU position 336 to EU position 348 and approximately from EU position 367 to EU position 393 and EU position 408 and approximately from EU position 424 to EU position 440. In one embodiment one or more of the following amino acid residues according to the EU numbering of Kabat are altered F243, P244, P245 P, K246, P247, K248, D249, T250, L251, M252, 1253, S254, R255, T256, P257, E258, V259, T260, C261, F275, N276, W277, Y278, V279, D280, V282, E283, V284, H285, N286, A287, K288, T289, K290, P291, R292, E293, V302, V303, S304, V305, L306, T307, V308, L309, H310, Q311, D312, G341, Q342, P343, R344, E345, P346, Q347, V348, C367, V369, F372, Y373, P374, S375, D376, 1377, A378, V379, E380, W381, E382, S383, N384, G385, Q386, P387, E388, N389, Y391, T393, S408, S424, C425, S426, V427, M428, H429, E430, A431, L432, H433, N434, H435, Y436, T437, Q438, K439, and S440 (EU numbering).

[0229] "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0230] The term "full length antibody" denotes an antibody having a structure substantially similar to a native antibody structure comprising four polypeptides or having heavy chains that contain an Fc-region as defined herein. A full length antibody may comprise further domains, such as e.g. a scFv or a scFab conjugated to one or more of the chains of the full length antibody. These conjugates are also encompassed by the term full length antibody.

[0231] The term "dimeric polypeptide" denotes a complex comprising at least two polypeptides that are associated covalently. The complex may comprise further polypeptides that are also associated covalently or non-covalently with the other polypeptides. In one embodiment the dimeric polypeptide comprises two or four polypeptides.

[0232] The terms "heterodimer" or "heterodimeric" denote a molecule that comprises two polypeptides (e.g. of comparable length), wherein the two polypeptides have an amino acid sequence that have at least one different amino acid residue in a corresponding position, whereby corresponding position is determined according to the Kabat EU index numbering system.

[0233] The terms "homodimer" and "homodimeric" denote a molecule that comprises two polypeptides of comparable length, wherein the two polypeptides have an amino acid sequence that is identical in corresponding positions, whereby corresponding positions are determined according to the Kabat EU index numbering system.

[0234] A dimeric polypeptide as reported herein can be homodimeric or heterodimeric which is determined with respect to mutations or properties in focus. For example, with respect to FcRn and/or protein A binding (i.e. the focused on properties) a dimeric polypeptide is homodimeric (i.e. both polypeptides of the dimeric polypeptide comprise these mutations) with respect to the mutations H310A, H433A and Y436A (these mutations are in focus with respect to FcRn and/or protein A binding property of the dimeric polypeptide) but at the same time heterodimeric with respect to the mutations Y349C, T366S, L368A and Y407V (these mutations are not in focus as these mutations are directed to the heterodimerization of the dimeric polypeptide and not to the FcRn/protein A binding properties) as well as the mutations S354C and T366W, respectively (the first set is comprised only in the first polypeptide whereas the second set is comprised only in the second polypeptide). Further for example, a dimeric polypeptide as reported herein can be heterodimeric with respect to the mutations I253A, H310A, H433A, H435A and Y436A (i.e. these mutations are directed all to the FcRn and/or protein A binding properties of the dimeric polypeptide), i.e. one polypeptide comprises the mutations I253A, H310A and H435A, whereas the other polypeptide comprises the mutations H310A, H433A and Y436A.

[0235] The terms "host cell", "host cell line", and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary

transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0236] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0237] A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat, E. A. et al., Sequences of Proteins of Immunological Interest, 5th ed., Bethesda Md. (1991), NIH Publication 91-3242, Vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the VH, the subgroup is subgroup

[0238] The term "derived from" denotes that an amino acid sequence is derived from a parent amino acid sequence by introducing alterations at at least one position. Thus a derived amino acid sequence differs from the corresponding parent amino acid sequence at at least one corresponding position (numbering according to Kabat EU index for antibody Fc-regions). In one embodiment an amino acid sequence derived from a parent amino acid sequence differs by one to fifteen amino acid residues at corresponding positions. In one embodiment an amino acid sequence derived from a parent amino acid sequence differs by one to ten amino acid residues at corresponding positions. In one embodiment an amino acid sequence derived from a parent amino acid sequence differs by one to six amino acid residues at corresponding positions. Likewise a derived amino acid sequence has a high amino acid sequence identity to its parent amino acid sequence. In one embodiment an amino acid sequence derived from a parent amino acid sequence has 80% or more amino acid sequence identity. In one embodiment an amino acid sequence derived from a parent amino acid sequence has 90% or more amino acid sequence identity. In one embodiment an amino acid sequence derived from a parent amino acid sequence has 95% or more amino acid sequence identity.

[0239] The term "human Fc-region polypeptide" denotes an amino acid sequence which is identical to a "native" or "wild-type" human Fc-region polypeptide. The term "variant (human) Fc-region polypeptide" denotes an amino acid sequence which derived from a "native" or "wild-type" human Fc-region polypeptide by virtue of at least one "amino acid alteration". A "human Fc-region" is consisting of two human Fc-region polypeptides. A "variant (human) Fc-region" is consisting of two Fc-region polypeptides, whereby both can be variant (human) Fc-region polypeptide and the other is a variant (human) Fc-region polypeptide.

[0240] In one embodiment the human Fc-region polypeptide has the amino acid sequence of a human IgG1 Fc-region polypeptide of SEQ ID NO: 60, or of a human IgG2

Fc-region polypeptide of SEQ ID NO: 61, or of a human IgG4 Fc-region polypeptide of SEQ ID NO: 63 with the mutations as reported herein. In one embodiment the variant (human) Fc-region polypeptide is derived from an Fc-region polypeptide of SEQ ID NO: 60, or 61, or 63 and has at least one amino acid mutation compared to the Fc-region polypeptide of SEQ ID NO: 60, or 61, or 63. In one embodiment the variant (human) Fc-region polypeptide comprises/has from about one to about ten amino acid mutations, and in one embodiment from about one to about five amino acid mutations. In one embodiment the variant (human) Fcregion polypeptide has at least about 80% homology with a human Fc-region polypeptide of SEQ ID NO: 60, or 61, or 63. In one embodiment the variant (human) Fc-region polypeptide has least about 90% homology with a human Fcregion polypeptide of SEQ ID NO: 60, or 61, or 63. In one embodiment the variant (human) Fc-region polypeptide has at least about 95% homology with a human Fc-region polypeptide of SEQ ID NO: 60, or 61, or 63.

[0241] The variant (human) Fc-region polypeptide derived from a human Fc-region polypeptide of SEQ ID NO: 60, or 61, or 63 is defined by the amino acid alterations that are contained. Thus, for example, the term P329G denotes a variant (human) Fc-region polypeptide derived human Fc-region polypeptide with the mutation of proline to glycine at amino acid position 329 relative to the human Fc-region polypeptide of SEQ ID NO: 60, or 61, or 63.

[0242] A human IgG1 Fc-region polypeptide has the following amino acid sequence:

(SEQ ID NO: 60)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED

PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK

 ${\tt CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK}$

 ${\tt GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG}$

 ${\tt NVFSCSVMHEALHNHYTQKSLSLSPGK}\,.$

[0243] A human IgG1 Fc-region derived Fc-region polypeptide with the mutations L234A, L235A has the following amino acid sequence:

(SEQ ID NO: 64)

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK

CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK

 ${\tt GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG}$

 ${\tt NVFSCSVMHEALHNHYTQKSLSLSPGK}\,.$

[0244] A human IgG1 Fc-region derived Fc-region polypeptide with Y349C, T366S, L368A and Y407V mutations has the following amino acid sequence:

(SEQ ID NO: 65)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED

 ${\tt PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK}$

CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVK

-continued

 $\label{thm:confidence} {\tt GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQG} $$ {\tt NVFSCSVMHEALHNHYTQKSLSLSPGK}.$

[0245] A human IgG1 Fc-region derived Fc-region polypeptide with S354C, T366W mutations has the following amino acid sequence:

(SEQ ID NO: 66)

 ${\tt DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED}$

 ${\tt PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK}$

CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK

GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG

NVFSCSVMHEALHNHYTQKSLSLSPGK.

[0246] A human IgG1 Fc-region derived Fc-region polypeptide with L234A, L235A mutations and Y349C, T366S, L368A, Y407V mutations has the following amino acid sequence:

(SEO ID NO: 67)

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK.

[0247] A human IgG1 Fc-region derived Fc-region polypeptide with a L234A, L235A and S354C, T366W mutations has the following amino acid sequence:

(SEQ ID NO: 68)

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK.

[0248] A human IgG1 Fc-region derived Fc-region polypeptide with a P329G mutation has the following amino acid sequence:

(SEQ ID NO: 69)

$$\label{thm:constraint} \begin{align} {\bf DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED} \\ {\bf PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK} \\ {\bf CKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK} \\ {\bf GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG} \\ {\bf CKVSNKALGAPIEKTISKAKGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG} \\ {\bf CKVSNKALGAPIEKTISKAKGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG \\ {\bf CKVSNKALGAPIEKTISKAKGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQG \\ {\bf CKVSNKALGAPIEKTISKAKGQPENNYKTPPVLDSDGSFFLYSKLTVDKSTWAGAPT \\ {\bf CKVSNKALGAPT \\ {\bf CKVSNKA$$

 ${\tt NVFSCSVMHEALHNHYTQKSLSLSPGK}.$

[0249] A human IgG1 Fc-region derived Fc-region polypeptide with L234A, L235A mutations and P329G mutation has the following amino acid sequence:

(SEQ ID NO: 70)

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK.

[0250] A human IgG1 Fc-region derived Fc-region polypeptide with a P239G mutation and Y349C, T366S, L368A, Y407V mutations has the following amino acid sequence:

(SEQ ID NO: 71)
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALGAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQG

[0251] A human IgG1 Fc-region derived Fc-region polypeptide with a P329G mutation and S354C, T366W mutation has the following amino acid sequence:

NVFSCSVMHEALHNHYTQKSLSLSPGK.

(SEQ ID NO: 72)
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK.

[0252] A human IgG1 Fc-region derived Fc-region polypeptide with L234A, L235A, P329G and Y349C, T366S, L368A, Y407V mutations has the following amino acid sequence:

(SEQ ID NO: 73)
DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALGAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK.

[0253] A human IgG1 Fc-region derived Fc-region polypeptide with L234A, L235A, P329G mutations and S354C, T366W mutations has the following amino acid sequence:

(SEQ ID NO: 74)
DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK

-continued

GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTOKSLSLSPGK.

[0254] A human IgG4 Fc-region polypeptide has the following amino acid sequence:

(SEQ ID NO: 63)
ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTOKSLSLSLGK.

[0255] A human IgG4 Fc-region derived Fc-region polypeptide with S228P and L235E mutations has the following amino acid sequence:

(SEQ ID NO: 75)
ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTOKSLSLSLGK.

[0256] A human IgG4 Fc-region derived Fc-region polypeptide with S228P, L235E mutations and P329G mutation has the following amino acid sequence:

(SEQ ID NO: 76) ESKYGPPCPPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLGSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLSLGK.

[0257] A human IgG4 Fc-region derived Fc-region polypeptide with S354C, T366W mutations has the following amino acid sequence:

(SEQ ID NO: 77)
ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKSLSLSLGK.

[0258] A human IgG4 Fc-region derived Fc-region polypeptide with Y349C, T366S, L368A, Y407V mutations has the following amino acid sequence:

EGNVFSCSVMHEALHNHYTQKSLSLSLGK.

(SEQ ID NO: 78)
ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCA
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSRWQ

[0259] A human IgG4 Fc-region derived Fc-region polypeptide with a S228P, L235E and S354C, T366W mutations has the following amino acid sequence:

(SEQ ID NO: 79)
ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKSLSLSLGK.

[0260] A human IgG4 Fc-region derived Fc-region polypeptide with a S228P, L235E and Y349C, T366S, L368A, Y407V mutations has the following amino acid sequence:

(SEQ ID NO: 80)
ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCA
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKSLSLSLGK.

[0261] A human IgG4 Fc-region derived Fc-region polypeptide with a P329G mutation has the following amino acid sequence:

(SEQ ID NO: 81)
ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLGSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTOKSLSLSLGK.

[0262] A human IgG4 Fc-region derived Fc-region polypeptide with a P239G and Y349C, T366S, L368A, Y407V mutations has the following amino acid sequence:

(SEQ ID NO: 82) ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLGSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLSLGK.

[0263] A human IgG4 Fc-region derived Fc-region polypeptide with a P329G and S354C, T366W mutations has the following amino acid sequence:

(SEQ ID NO: 83)
ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLGSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTOKSLSLSLGK.

[0264] A human IgG4 Fc-region derived Fc-region polypeptide with a S228P, L235E, P329G and Y349C, T366S, L368A, Y407V mutations has the following amino acid sequence:

(SEQ ID NO: 84)
ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLGSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCA
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKSLSLSLGK.

[0265] A human IgG4 Fc-region derived Fc-region polypeptide with a S228P, L235E, P329G and S354C, T366W mutations has the following amino acid sequence:

(SEQ ID NO: 85) ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLGSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLSLGK.

[0266] An alignment of the different human Fc-regions is shown below (Kabat EU index numbering system):

-continued 2 5 IGG1 DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED ...VECPPCP APP.VAGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED IGG2 IGG3 DTPPPCPRCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED ...PPCPSCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED IGG4 -- HINGE - | -- CH2 -----3 0 IGG1 PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK PEVQFNWYVD GVEVHNAKTK PREEQFNSTF RVVSVLTVVH QDWLNGKEYK IGG2 PEVOFKWYVD GVEVHNAKTK PREEQYNSTF RVVSVLTVLH ODWLNGKEYK IGG3 PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK IGG4 5 0 TGG1 CKVSNKALPA PIEKTISKAK GOPREPOVYT LPPSRDELTK NOVSLTCLVK IGG2 CKVSNKGLPA PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK CKVSNKALPA PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK TGG3 IGG4 CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK -- CH2 ----- CH2 -- -- CH3 -----0 IGG1 GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG IGG2 GFYPSDISVE WESNGQPENN YKTTPPMLDS DGSFFLYSKL TVDKSRWQQG IGG3 GFYPSDIAVE WESSGQPENN YNTTPPMLDS DGSFFLYSKL TVDKSRWQQG GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG IGG4 -- CH3 -----4 IGG1 NVFSCSVMHE ALHNHYTQKS LSLSPGK IGG2 NVFSCSVMHE ALHNHYTQKS LSLSPGK NIFSCSVMHE ALHNRFTQKS LSLSPGK NVFSCSVMHE ALHNHYTQKS LSLSLGK IGG4

[0267] A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., the CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

[0268] The term "hypervariable region" or "HVR", as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence ("complementarity determining regions" or "CDRs") and form structurally defined loops ("hypervariable loops"), and/or contain the antigen-contacting residues ("antigen contacts"). Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs as denoted herein include

[0269] (a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia, C. and Lesk, A. M., J. Mol. Biol. 196 (1987) 901-917);

[0270] (b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat, E. A. et al., Sequences of Proteins of Immunological Interest, 5th ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), NIH Publication 91-3242.);

[0271] (c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. J. Mol. Biol. 262: 732-745 (1996)); and

[0272] (d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

[0273] Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to the Kabat EU index numbering system (Kabat et al., supra).

[0274] The term "IGF-1R" as used herein, refers to any native IGF-1R from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length", unprocessed IGF-1R as well as any form of IGF-1R that results from processing in the cell. The term also encompasses naturally occurring variants of IGF-1R,

e.g., splice variants or allelic variants. The amino acid sequence of human IGF-1R is shown in SEQ ID NO: 11. [0275] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g. cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

[0276] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., size exclusion chromatography, ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman, S. et al., J. Chrom. B 848 (2007) 79-87. [0277] An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal

[0278] "Isolated nucleic acid encoding an anti-IGF-1R antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[0279] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described

[0280] "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three

constant domains (CH1, CH2, and CH3). Similarly, from N-to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

[0281] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0282] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, Calif., or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4. 0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0283] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence

identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0284] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0285] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0286] The term "peptidic linker" as used herein denotes a peptide with amino acid sequences, which is in one embodiment of synthetic origin. The peptidic linker is in one embodiment a peptide with an amino acid sequence with a length of at least 30 amino acids, in one embodiment with a length of 32 to 50 amino acids. In one embodiment the peptidic linker is a peptide with an amino acid sequence with a length of 32 to 40 amino acids. In one embodiment the peptidic linker is $(G \times S)n$ with G = glycine, S = serine, S = se

[0287] The term "recombinant antibody", as used herein, denotes all antibodies (chimeric, humanized and human) that are prepared, expressed, created or isolated by recombinant means. This includes 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 can be 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.

[0288] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies or Fc-region fusion polypeptides as reported herein are used to delay development of a disease or to slow the progression of a disease.

[0289] The term "valent" as used within the current application denotes the presence of a specified number of binding sites in a (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 a (antibody) molecule. The bispecific antibodies as reported herein are in one preferred embodiment "bivalent".

[0290] The term "variable region" or "variable domain" refer to the domain of an antibody heavy or light chain that is involved in binding of the antibody to its antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of an antibody generally have similar structures, with each domain comprising four framework regions (FRs) and three hypervariable regions (HVRs) (see, e.g., Kindt, T. J. et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., N.Y. (2007), page 91). A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively (see, e.g., Portolano, S. et al., J. Immunol. 150 (1993) 880-887; Clackson, T. et al., Nature 352 (1991) 624-628).

[0291] The term "ocular vascular disease" includes, but is 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, agerelated macular degeneration, retinitis pigmentosa, retinal angiomatous proliferation, macular telangectasia, ischemic retinopathy, iris neovascularization, intraocular neovascularization, corneal neovascularization, retinal neovascularization, choroidal neovascularization, and retinal degeneration (see e.g. 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).

[0292] The term "vector", as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors".

[0293] 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: 30; 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) 1306-1309; Houck et al., Mol. Endocrin. 5 (1991) 1806-1814; Keck, P. J., et al., Science 246 (1989) 1309-1312 and Connolly, D. T., et al., J. Biol. Chem. 264 (1989) 20017-20024; 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., Endocrin. 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.

[0294] The term "with (the) mutation IHH-AAA" as used herein refers to the combination of the mutations I253A (Ile253Ala), H310A (His310Ala), and H435A (His435Ala) and the term "with (the) mutation HF-TY-AAA" as used herein refers to the combination of the mutations H310A (His310Ala), H433A (His433Ala), and Y436A (Tyr436Ala) and the term "with (the) mutation YTE" as used herein refers to the combination of mutations M252Y (Met252Tyr), S254T (Ser254Thr), and T256E (Thr256Glu) in the constant heavy chain region of IgG1 or IgG4 subclass, wherein the numbering is according to the Kabat EU index numbering system.

[0295] The term "with (the) mutations P329G LALA" as used herein refers to the combination of 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 Kabat EU index numbering system. The term "with (the) mutation SPLE" as used herein refers to the combination of the mutations S228P (Ser228Pro) and L235E (Leu235Glu) in the constant heavy chain region of IgG4 subclass, wherein the numbering is according to the Kabat EU index numbering system. The term "with (the) mutation SPLE and P329G" as used herein refers to the combination of the mutations S228P (Ser228Pro), L235E (Leu235Glu) and P329G (Pro329Gly) in the constant heavy chain region of IgG4 subclass, wherein the numbering is according to the Kabat EU index numbering system.

II. Compositions and Methods

[0296] In one aspect, the invention is based, in part, on the finding that specific mutations or combination of mutations which influence the binding of an immunoglobulin Fcregion to the neonatal Fc-receptor (FcRn), i.e. which reduce or even eliminate the binding of the Fc-region to FcRn, do not simultaneously eliminate the binding of the Fc-region to Staphylococcal protein A. This has a profound effect on the purification process that can be employed as e.g. no specific and species limited affinity chromatography materials, such as e.g. KappaSelect which only binds to antibodies comprising a kappa light chain, are required. Thus, with the combination of mutations as reported herein it is possible at the same time to reduce or even eliminate the binding to FcRn while maintaining the binding to Staphylococcal protein A

[0297] In one aspect, the invention is based, in part, on the finding that by using different mutations in the Fc-regions of each heavy chain of a heterodimeric molecule, such as e.g. a bispecific antibody, can be provided that on the one hand

has a reduced or even eliminated binding to FcRn but on the other hand maintains the ability to bind to Staphylococcal protein A. This binding to Staphylococcal protein A can be used to separate the heterodimeric molecule from homodimeric by-products. For example by combining the mutations I253A, H310A and H435A in one heavy chain Fc-region with the mutations H310A, H433A and Y436A in the other heavy chain Fc-region using the knobs-into-hole approach a heterodimeric Fc-region can be obtained that on the one hand does not bind to FcRn (both sets of mutations are silent with respect to the human FcRn) but maintains binding to Staphylococcal protein A (the heavy chain Fc-region with the mutations I253A, H310A and H435A does not bind to FcRn and does not bind to Staphylococcal protein A, whereas the heavy chain Fc-region with the mutations H310A, H433A and Y436A does not bind to FcRn but does still bind to Staphylococcal protein A). Thus, standard protein A affinity chromatography can be used to remove the homodimeric hole-hole by-product as this no longer binds to Staphylococcal protein A). Thus, by combining the knobsinto-holes approach with the mutations I253A, H310A and H435A in the hole chain and the mutations H310A, H433A and Y436A in the knobs chain the purification/separation of the heterodimeric knobs-into-holes product from the homodimeric hole-hole by-product can be facilitated.

[0298] In one aspect, the invention is based, in part, on the finding that antibodies for intravitreal application are beneficial that do not have FcRn-binding as these antibodies can cross the blood-retinal-barrier, do not have substantially prolonged or shortened half-lives in the eye and are cleared fast from the blood circulation resulting in no or very limited systemic side effects outside the eye. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of ocular vascular diseases.

[0299] The invention is based, at least in part, on the finding that by using different mutations in each of the Fc-region polypeptides of an Fc-region a heterodimeric molecule, such as e.g. a bispecific antibody, can be provided that has tailor-made FcRn-binding and therewith antibodies can be provided that have a tailor-made systemic half-life. [0300] The combination of mutations I253A, H310A, H435A, or L251D, L314D, L432D, or L251S, L314S, L432S result in a loss of the binding to protein A, whereas the combination of mutations I253A, H310A, H435A, or H310A, H433A, Y436A, or L251D, L314D, L432D result in a loss of the binding to the human neonatal Fc receptor.

[0301] The following table presents an exemplary overview of the amino acid residues in an Fc-region that are involved in interactions or have been changed to modify interactions.

	interaction with			KiH protein A		effect of mutations on	
residue	protein A	FcRn	knob	hole	binding	FcRn binding	
Pro238						P238A increase	
Thr250						T250Q/M428L increase	
Leu251	main-chain						
	contact						
Met252	hydrophobic					M252W increase;	
	packing					M252Y increase;	
						M252Y/T256Q increase	
						M252F/T256D increase;	
						M252Y/S254T/T256E	
						increase	

-continued

	interaction	on with]	KiH	_protein A	effect of mutations on
residue	protein A	FcRn	knob	hole	binding	FcRn binding
Ile253	main-chain contact; hydrogen bonding; significant binding reduction if	interaction				I253A reduction
Ser254	mutated to Ala polar interaction; hydrogen bonding					S254A reduction; M252Y/S254T/T256E increase
Arg255 Thr256	salt-bridge					R255A reduction T256A increase; T256Q increase; T256P increase; M252Y/T256Q reduction; M252F/T256D reduction; M252Y/S254T/T256E
Pro257						increase P257I/Q311I increase;
Glu272 Asp280 His285 Lys288						P257I/N434H increase E272A increase D280K increase reduction K288A reduction;
Val305 Thr307						K288A/N434A increase V305A increase T307A increase; T307A/E380A/N434A increase; T307Q/N434A increase; T307Q/N434S increase; T307Q/E380A/N434A
Val308 Leu309 His310		interaction				increase V308P/N434A increase L309A reduction H310A reduction; H310Q/H433N
Gln311	polar or charged interaction					reduction Q311A increase; P257I/Q311I increase
Asp312 Leu314	hydrophobic interaction					D312A increase
Lys317 Ala339 Tyr349 Ser354 Thr366 Leu368			S354C T366W	Y349C T366S L368A		K317A increase A339T increase
Asp376				LJUGA		D376A increase; D376V/N434H increase
Ala378 Glu380	salt-bridge					A378Q increase E380A increase E380A/N434A increase; T307A/E380A/N434A increase; T307Q/E380A/N434A increase
Glu382 Gly385						E382A increase G385H increase; G385A/Q386P/N389S increase
Gln386						G385A/Q386P/N389S increase
Asn389						G385A/Q386P/N389S increase

-continued

	interaction	n with	KiH		_protein A	effect of mutations on	
residue	protein A	FcRn	knob	hole	binding	FcRn binding	
Tyr407 Ser415 Ser424 Met428 Leu432	polar or			Y407V		S415A reduction S424A increase M428L increase; T250Q/M428L increase	
His433	charged interaction polar or charged interaction; salt-bridge	interaction				H433A reduction; H310Q/H433N reduction; H433K/N434F/Y436Hincrease; H433R/N434Y/Y436Hincrease;	
Asn434	hydrogen bonding; significant binding reduction if replaced by Ala	interaction				H433K/N434F increase N434W/Y/F/A/H increase; K288A/N434A increase; E380A/N434A increase; T307A/E380A/N434A increase; H433K/N434F/Y436H increase; H433K/N434F/Y436Hincrease; H433K/N434F/Y436Hincrease; H433K/N434F increase; D376V/N434H increase; T307Q/N434H increase; T307Q/N434A increase; T307Q/N434A increase; T307Q/N434A increase; T307Q/E380A/N434A increase;	
His435	hydrophobic packing; significant binding reduction if mutated to Ala	interaction			H435R/Y436F eliminates binding to protein A	H435R reduction; H435R reduction	
Tyr436	hydrophobic packing; significant binding reduction if replaced by Ala	interaction			H435R/Y436F eliminates binding to protein A	Y436A reduction; N434F/Y436H increase; H433K/N434F/Y436Hincrease; H433R/N434Y/Y436H increase	

[0302] The modifications as reported herein alter the binding specificity for one or more Fc receptors such as the human FcRn. At the same time some of the mutations which alter the binding to human FcRn do not alter the binding to Staphylococcal protein A.

[0303] In one embodiment the combination of mutations as reported herein does alter or does substantially alter the serum half-life of the dimeric polypeptide as compared with a corresponding dimeric polypeptide that lacks this combination of mutations. In one embodiment the combination of mutations further does not alter or does not substantially alter the binding of the dimeric polypeptide to protein A as compared with a corresponding dimeric polypeptide that lacks this combination of mutations.

A. The Neonatal Fe-Receptor (FcRn)

[0304] The neonatal Fc-receptor (FcRn) is important for the metabolic fate of antibodies of the IgG class in vivo. The FcRn functions to salvage wild-type IgG from the lysosomal degradation pathway, resulting in reduced clearance and increased half-life. It is a heterodimeric protein consisting of

two polypeptides: a 50 kDa class I major histocompatibility complex-like protein (α -FcRn) and a 15 kDa β 2-microglobulin (β 2m). FcRn binds with high affinity to the CH2-CH3 portion of the Fc-region of an antibody of the class IgG. The interaction between an antibody of the IgG class and the FcRn is pH dependent and occurs in a 1:2 stoichiometry, i.e. one IgG antibody molecule can interact with two FcRn molecules via its two heavy chain Fc-region polypeptides (see e.g. Huber, A. H., et al., J. Mol. Biol. 230 (1993) 1077-1083).

[0305] Thus, an IgGs in vitro FcRn binding properties/ characteristics are indicative of its in vivo pharmacokinetic properties in the blood circulation.

[0306] In the interaction between the FcRn and the Fcregion of an antibody of the IgG class different amino acid residues of the heavy chain CH2- and CH3-domain are participating. The amino acid residues interacting with the FcRn are located approximately between EU position 243 and EU position 261, approximately between EU position 275 and EU position 293, approximately between EU position 302 and EU position 319, approximately between EU

position 336 and EU position 348, approximately between EU position 367 and EU position 393, at EU position 408, and approximately between EU position 424 and EU position 440. More specifically the following amino acid residues according to the EU numbering of Kabat are involved in the interaction between the Fc-region and the FcRn: F243, P244, P245 P, K246, P247, K248, D249, T250, L251, M252, 1253, S254, R255, T256, P257, E258, V259, T260, C261, F275, N276, W277, Y278, V279, D280, V282, E283, V284, H285, N286, A287, K288, T289, K290, Q311, D312, W313, L314, N315, G316, K317, E318, Y319, I336, S337, K338, A339, K340, G341, Q342, P343, R344, E345, P346, Q347, V348, C367, V369, F372, Y373, P374, S375, D376, 1377, A378, V379, E380, W381, E382, S383, N384, G385, Q386, P387, E388, N389, Y391, T393, S408, S424, C425, S426, V427, M428, H429, E430, A431, L432, H433, N434, H435, Y436, T437, Q438, K439, and S440.

[0307] Site-directed mutagenesis studies have proven that the critical binding sites in the Fc-region of IgGs for FcRn are Histidine 310, Histidine 435, and Isoleucine 253 and to a lesser extent Histidine 433 and Tyrosine 436 (see e.g. Kim, J. K., et al., Eur. J. Immunol. 29 (1999) 2819-2825; Raghavan, M., et al., Biochem. 34 (1995) 14649-14657; Medesan, C., et al., J Immunol. 158 (1997) 2211-2217).

[0308] Methods to increase IgG binding to FcRn have been performed by mutating IgG at various amino acid residues: Threonine 250, Methionine 252, Serine 254, Threonine 256, Threonine 307, Glutamic acid 380, Methionine 428, Histidine 433, and Asparagine 434 (see Kuo, T. T., et al., J. Clin. Immunol. 30 (2010) 777-789).

[0309] In some cases antibodies with reduced half-life in the blood circulation are desired. For example, drugs for intravitreal application should have a long half-live in the eye and a short half-life in the blood circulation of the patient. Such antibodies also have the advantage of increased exposure to a disease site, e.g. in the eye.

[0310] Different mutations that influence the FcRn binding and therewith the half-live in the blood circulation are known. Fc-region residues critical to the mouse Fc-regionmouse FcRn interaction have been identified by site-directed mutagenesis (see e.g. Dall'Acqua, W. F., et al. J. Immunol 169 (2002) 5171-5180). Residues I253, H310, H433, N434, and H435 (EU numbering according to Kabat) are involved in the interaction (Medesan, C., et al., Eur. J. Immunol. 26 (1996) 2533-2536; Firan, M., et al., Int. Immunol. 13 (2001) 993-1002; Kim, J. K., et al., Eur. J. Immunol. 24 (1994) 542). Residues I253, H310, and H435 were found to be critical for the interaction of human Fc with murine FcRn (Kim, J. K., et al., Eur. J. Immunol. 29 (1999) 2819-2855). Residues M252Y, S254T, T256E have been described by Dall'Acqua et al. to improve FcRn binding by proteinprotein interaction studies (Dall'Acqua, W. F., et al. J. Biol. Chem. 281 (2006) 23514-23524). Studies of the human Fc-human FcRn complex have shown that residues I253, S254, H435, and Y436 are crucial for the interaction (Firan, M., et al., Int. Immunol. 13 (2001) 993-1002; Shields, R. L., et al., J. Biol. Chem. 276 (2001) 6591-6604). In Yeung, Y. A., et al. (J. Immunol. 182 (2009) 7667-7671) various mutants of residues 248 to 259 and 301 to 317 and 376 to 382 and 424 to 437 have been reported and examined. Exemplary mutations and their effect on FcRn binding are listed in the following Table.

TABLE

	** ***		
mutation	effect on FcRn binding	half-live in the circulation	reference
H285 H310Q/H433N (murine IgG1)	reduced (murine)	reduced (in mouse)	Kim, J. K., Scand. J. Immunol. 40 (1994) 457-465
I253A H310A H435A H436A	reduced (murine)	reduced (in mouse)	Ghetie, V. and Ward, E. S., Immunol. Today 18 (1997) 592-598
(murine IgG1) T252L/T254S/T256F T252A/T254S/T256A (murine IgG1)	increased (murine)	increased (in mouse)	Ghetie, V. and Ward, E. S., Immunol. Today 18 (1997) 592-598
I253A H310A H435A H436A	reduced (murine)	reduced (in mouse)	Medesan, C., et al., J. Immunol. 158 (1997) 2211-2217
H433A/N434Q (murine IgG1) I253A H310A H435A H435R	reduced H310A: <0.1 rel. binding to muFcRn	reduced (in mouse)	Kim, J. K., Eur. J. Immunol. 29 (1999) 2819-2825
(human IgG1) H433A (human IgG1)	(murine) 1.1 rel. binding to muFcRn, 0.4 rel. binding hu FcRn (murine)		Kim, J. K., Eur. J. Immunol. 29 (1999) 2819-2825
I253A S254A H435A Y436A (human IgG1)	reduced <0.1 relative binding to huFcRn	reduced	Shields, R. L., et al., J. Biol. Chem. 276 (2001) 6591-6604

TABLE-continued

	IABLE-	continued	
mutation	effect on FcRn binding	half-live in the circulation	reference
R255A	reduced	reduced	Shields, R. L., et al.,
K288A	(human)	redirect	J. Biol. Chem. 276
L309A	()		(2001) 6591-6604
S415A			
H433A			
(human IgG1)			
P238A	increased	increased	Shields, R. L., et al.,
T256A	(human)		J. Biol. Chem. 276
E272A			(2001) 6591-6604
V305A			
T307A			
Q311A			
D312A K317A			
D376A			
A378Q			
E380A			
E382A			
S424A			
N434A			
K288A/N434A			
E380A/N434A			
T307A/E380A/N434A			
(human IgG1)	1 1 0 4	, ,	
H435A	reduced <0.1	reduced	Firan, M., et al.,
(humanized IgG1)	rel. binding to huFcRn		Int. Immunol. 13 (2001) 993-1002
I253A (no binding)	increased	reduced	Dall'Acqua,
M252W	(murine and	(in mouse)	J. Immunol. 169
M252Y	human)	(III IIIeuse)	(2002) 5171-5180
M252Y/T256Q	,		(===, =====
M252F/T256D			
N434F/Y436H			
M252Y/S254T/T256E			
G385A/Q386P/N389S			
H433K/N434F/Y436H			
H433R/N434Y/Y436H			
G385R/Q386T/P387R/N389P M252Y/S254T/T256E/H433K/			
N434F/Y436H			
M252Y/S254T/T256E/G385R/			
Q386T/P387R/N389P			
(human IgG1)			
M428L	increased	increased	Hinton, P. R., et al.,
T250Q/M428L	(human)	(in monkey)	J. Biol. Chem. 279
(human IgG2)			(2004) 6213-6216
M252Y/S254T/T256E +	increased	increased	Vaccaro, C., et al.,
H433K/N434F	(human)	(in mouse)	Nat. Biotechnol. 23
(human IgG)			(2005) 1283-1288
T307A/E380A/N434A	increased	increased in	Pop, L. M., et al.,
(chimeric IgG1)		transgenic mouse	Int. Immunopharmacol. 5 (2005) 1279-1290
T250Q	increased	increased in	Petkova, S. B., et al.,
E380A	(human)	transgenic mouse	Int. Immunol 18
M428L	()	8	(2006) 1759-1769
N434A			,
K288A/N434A			
E380A/N434A			
T307A/E380A/N434A			
(human IgG1)			
I253A	reduced	reduced in	Petkova, S. B., et al.,
(human IgG1)	(human)	transgenic mouse	Int. Immunol 18
S239D/A330L/I332E	increased	increased in	(2006) 1759-1769 Dall'Acqua, W. F., et
M252Y/S254T/T256E	(human and	Cynomolgus	al., J. Biol. Chem. 281
(humanized)	Cynomolgus)	Symomorgus	(2006) 23514-23524
T250Q	increased	increased in	Hinton, P. R., et al.,
M428L	(human)	Rhesus apes	J. Immunol. 176
T250Q/M428L	` -7	r	(2006) 346-356
(human IgG1)			
T250Q/M428L	increased	no change in	Datta-Mannan, A., et
P257I/Q311I	(mouse and	Cynomolgus	al., J. Biol. Chem. 282
(humanized IgG1)	Cynomolgus)	increased in mouse	(2007) 1709-1717
P257I/Q311I	increased	reduced in mice	Datta-Mannan, A., et

TABLE-continued

mutation	effect on FcRn binding	half-live in the circulation	reference
P257I/N434H D376V/N434H (humanized IgG1)	at pH 6 (human, Cynomolgus,	P257I/N434H reduced in Cynomolgus	al., Drug Metab. Dispos. 35 (2007) 86-94
abrogate FcRn binding: 1253 H310 H433 H435 reduce FcRn binding: Y436 increased FcRn binding: T250 N252 S254 T256 T307 M428 N434	mouse) increased and reduced	reducing the binding ability of IgG for FcRn reduces its serum persistence; a higher-affinity FcRn-IgG interaction prolongs the half-lives of IgG and Fc-coupled drugs in the serum	Ropeenian, D. C. and Akilesh, S., Nat. Rev. Immunol. 7 (2007) 715-725
N434A T307Q/N434A T307Q/N434S V308P/N434A T307Q/E380A/N434A	increased (Cynomolgus monkey)	increased in Cynomolgus monkey	Yeung, Y. A., et al., Cancer Res. 70 (2010) 3269-3277
(human IgG1) 256P 280K 339T 385H 428L 434W/Y/F/A/H (human IgG)	increased at neutral pH		WO 2011/122011

[0311] It has been found that one mutation one-sided in one Fc-region polypeptide is sufficient to weaken the binding significantly. The more mutations are introduced into the Fc-region the weaker the binding to the FcRn becomes. But one-sided asymmetric mutations are not sufficient to completely inhibit FcRn binding. Mutations on both sides are necessary to completely inhibit FcRn binding.

[0312] The results of a symmetric engineering of an IgG1 Fc-region to influence FcRn binding is shown in the following table (alignment of mutations and retention time on an FcRn-affinity chromatography column).

TABLE

effector function influencing mutations	FcRn- binding influencing mutation 1	FcRn- binding influencing mutation 2	FcRn- binding influencing mutation 3	FcRn- affinity column retention time [min]
L234A/L235A/ P329G	_	_	_	45.3
L234A/L235A/ P329G	I253A	H310A	H435A	2.3
L234A/L235A/ P329G	I253A	_	_	2.7
L234A/L235A/ P329G	_	H310A	_	2.4
L234A/L235A/ P329G	_	_	H435A	2.7
L234A/L235A/ P329G	I253A	H310A	_	2.3
L234A/L235A/ P329G	I253A	_	H435A	2.3

TABLE-continued

effector function influencing mutations	FcRn- binding influencing mutation 1	FcRn- binding influencing mutation 2	FcRn- binding influencing mutation 3	FcRn- affinity column retention time [min]
L234A/L235A/ P329G	_	H310A	H435A	2.4
L234A/L235A/ P329G	_	H310A	Y436A	2.3
L234A/L235A/ P329G	H310A	H433A	Y436A	2.4
L234A/L235A/ P329G	_	_	Y436A	41.3

[0313] Retention times below 3 minutes correspond to no binding as the substance is in the flow-through (void peak).
[0314] The single mutation H310A is the most silent symmetrical mutation to delete any FcRn-binding.

[0315] The symmetric single mutation I253A and H435A result in a relative shift of retention time of 0.3 to 0.4 min. This can be generally regarded as a non-detectable binding.

[0316] The single mutation Y436A results in detectable interaction strength to the FcRn affinity column. Without being bound by this theory this mutation could have an effect on FcRn mediated in vivo half-life which can be differentiated from a zero interaction such as the combination of the I253A, H310A and H435A mutations (IHH-AAA mutation).

[0317] The results obtained with a symmetrically modified anti-HER2 antibody are presented in the following table (see WO 2006/031370 for reference).

TABLE

mutation	retention time [min]
I253H	no binding
M252D	no binding
S254D	no binding
R255D	41.4
M252H	43.6
K288E	45.2
L309H	45.5
E258H	45.6
T256H	46.0
K290H	46.2
D98E	46.2
wild-type	46.3
K317H	46.3
Q311H	46.3
E430H	46.4
T307H	47.0
N434H	52.0

[0318] The effect of the introduction of asymmetric FcRn-binding affecting mutations in the Fc-region has been exemplified with a bispecific antibody assembled using the knobs-into-holes technology (see e.g. U.S. Pat. No. 7,695,936, US 2003/0078385; "hole chain" mutations: S354C/T366W, "knob chain" mutations: Y349C/T366S/L368A/Y407V). The effect of the asymmetrically introduced mutations on FcRn-binding can easily be determined using an FcRn affinity chromatography method (see FIG. 9 and the following Table). Antibodies that have a later elution from the FcRn affinity column, i.e. that have a longer retention time on the FcRn affinity column, have a longer half-life in vivo, and vice versa.

TABLE

FcRn affecting mutation	retention time on FcRn affinity column
one chain with M252Y/ S254T/T256E	56.2 min.
none	51.8 min.
one chain with I253A or H435A	48.8 min.
one chain with H310A	48.4 min.
one chain with I253A/	48.0 min.
H435A or I253A/H310A or H310A/H435A	
one chain with H310A/ H433A/Y436A	46.7 min.
one chain with I253A/	46.6 min.
one chain with L251D/ L314D/L432D	46.3 min.
first chain with I253A/ H310A/H435A and second chain with H310A or H435A or I253A/H310A/H435A	no binding

[0319] The effect of the introduction of asymmetric FcRn-binding affecting mutations in the Fc-region has further been exemplified with a monospecific anti-IGF-1R antibody assembled using the knobs-into-holes technology in order to allow the introduction of asymmetric mutations (see e.g. U.S. Pat. No. 7,695,936, US 2003/0078385; "hole chain" mutations: S354C/T366W, "knob chain" mutations: Y349C/T366S/L368A/Y407V). The effect of the asymmetrically introduced mutations on FcRn-binding can easily be determined using an FcRn affinity chromatography method (see the following Table). Antibodies that have a later elution from the FcRn affinity column, i.e. that have a longer retention time on the FcRn affinity column, have a longer half-life in vivo, and vice versa.

TABLE

FcRn affecting mutation	retention time on FcRn affinity column
one chain with M252Y/ S254T/T256E	57.6 min.
none	53.0 min.
one chain with H310A/ H433A/Y436A	42.4 min.
one chain with I253A/ H310A/H435A	42.0 min.
one chain with L251D/ L314D/L432D	40.9 min.
first chain with I253A/ H310A/H435A and second chain with H310A or H435A or I253A/H310A/H435A	no binding

[0320] The asymmetric IHH-AAA and LLL-DDD mutation (LLL-DDD-mutation=combination of the mutations L251D, L314D and L432D) show weaker binding than the corresponding parent or wild-type antibody.

[0321] The symmetric HHY-AAA mutation (=combination of the mutations H310A, H433A and Y436A) results in an Fc-region that does no longer bind to the human FcRn whereas the binding to protein A is maintained (see FIGS. 11, 12, 13 and 14).

[0322] The effect of the introduction of asymmetric FcRn-binding affecting mutations in the Fc-region has further been exemplified with a monospecific anti-IGF-1R antibody (IGF-1R), a bispecific anti-VEGF/ANG2 antibody (VEGF/ANG2), and a full length antibody with fusions to the C-terminus of both heavy chains (fusion) assembled using the knobs-into-holes technology in order to allow the introduction of asymmetric mutations (see e.g. U.S. Pat. No. 7,695,936, US 2003/0078385; "hole chain" mutations: S354C/T366W, "knob chain" mutations: Y349C/T366S/L368A/Y407V). The effect of the introduced mutations on FcRn-binding and protein A binding can easily be determined using an FcRn affinity chromatography method, a protein A affinity chromatography method and SPR-based methods (see the following Table).

antibody	further mutation in knob chain	further mutation in hole chain	FcR binding affecting mutations	FcRn binding (SPR)	FcRn binding (column)	protein A binding (SPR)	protein A binding (column)
VEGF/ ANG2 0096	none	none	L234A L235A P329G	yes	yes	stable binding	yes

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-continued										
antika de	further mutation in knob	further mutation in hole	FcR binding affecting mutations	FcRn binding	FcRn binding	protein A binding	protein A			
antibody	chain	chain	mutations	(SPR)	(column)	(SPR)	(column)			
VEGF/	none	I253A	L234A	yes	yes	fast off-	yes			
ANG2		H310A	L235A			rate				
0097		H435A	P329G							
VEGF/	none	H310A	L234A	yes	yes	stable	yes			
ANG2		H433A	L235A			binding				
0098		Y436A	P329G							
VEGF/	none	L251D	L234A	reduced	reduced	fast off-	yes			
ANG2		L314D	L235A			rate				
0099		L432D	P329G							
VEGF/	none	M252Y	L234A	in-	in-	n.d.	yes			
ANG2		S254T	L235A	creased	creased					
0100	T252.4	T256E	P329G	1		1				
VEGF/	I253A	I253A	L234A	n.d.	no	n.d.	no			
ANG2	H310A	H310A	L235A							
0016	H435A	H435A	P329G	1	1	1				
VEGF/	H310A	H310A	L234A	n.d.	n.d.	n.d.	yes			
ANG2	H433A	H433A	L235A							
0121 GE 1D	Y436A	Y436A	P329G	*****	*****	n d	*****			
GF-1R	none	none	none	yes	yes	n.d.	yes			
0033 IGF-1R	none	1252 4	T 2244	n d	Mod	n d	TIOC			
	none	I253A H310A	L234A L235A	n.d.	yes	n.d.	yes			
0034		H310A	L233A P329G							
ICE 1D	none	H435A		noduce d	mada a a d	n d	Tion			
IGF-1R	none	H310A	none	reduced	reduced	n.d.	yes			
0035		H433A								
OF 45		Y436A	* * * * * * * * * * * * * * * * * * * *							
IGF-1R	none	L251D	L234A	n.d.	yes	n.d.	yes			
0037		L314D	L235A							
		L432D	P329G							
GF-1R	none	M252Y	L234A	n.d.	yes	n.d.	yes			
0036		S254T	L235A							
		T256E	P329G							
GF-1R	H310A	H310A	none	n.d.	n.d.	n.d.	yes			
0045	H433A	H433A								
	Y436A	Y436A								
fusion	none	none	L234A	n.d.	yes	n.d.	n.d.			
0008			L235A		J					
			P329G							
fusion	I253A	I253A	L234A	n.d.	no	n.d.	n.d.			
1081011 0019	12.55A	1233A	L234A L235A	п.ч.	но	n.u.	п.ч.			
ハリスプ										
G. a.l. c	112101	TT210 *	P329G	m .d						
fusion	H310A	H310A	L234A	n.d.	no	n.d.	n.d.			
0020			L235A							
			P329G							
fusion	H435A	H435A	L234A	n.d.	no	n.d.	n.d.			
0021			L235A							
			P329G							
fusion	Y436A	Y436A	L234A	n.d.	reduced	n.d.	n.d.			
0038			L235A							
			P329G							
fusion	I253A	I253A	L234A	n.d.	no	n.d.	n.d.			
0022	H310A	H310A	L235A							
			P329G							
fusion	I253A	I253A	L234A	n.d.	no	n.d.	n.d.			
0023				m.d.	110	11.0.	11.4.			
1023	H435A	H435A	L235A							
	TT210.	TT210 *	P329G	1						
usion	H310A	H310A	L234A	n.d.	no	n.d.	n.d.			
0036	H435A	H435A	L235A							
			P329G							
fusion	H310A	H310A	L234A	n.d.	no	n.d.	n.d.			
0037	Y436A	Y436A	L235A							
			P329G							
		I253A	L234A	n.d.	no	n.d.	n.d.			
usion	I253A									
			L235A							
	H310A	H310A	L235A P329G							
fusion 0018	H310A H435A	H310A H435A	P329G	n.d.	no	n.d.	n.d.			
	H310A	H310A		n.d.	no	n.d.	n.d.			

[0323] One aspect as reported herein is an antibody or Fc-region fusion polypeptide comprising the variant human IgG class Fc-region as reported herein.

[0324] The Fc-region (dimeric polypeptide) as reported herein when contained in an Fc-region fusion polypeptide or a full length antibody confers the above described characteristics to the molecule. The fusion partner can be any molecules having a biological activity who's in vivo half-live shall be reduced or increased, i.e. who's in vivo half-live shall be clearly defined and tailor-made for its intended application.

[0325] Fc-region fusion polypeptides may comprise e.g. a variant (human) IgG class Fc-region as reported herein and a receptor protein that binds to a target including a ligand, such as, for example, TNFR-Fc-region fusion polypeptide (TNFR=human tumor necrosis factor receptor), or IL-1R-Fc-region fusion polypeptide (IL-1R=human interleukin-1 receptor), or VEGFR-Fc-region fusion polypeptides (VEGFR=human vascular endothelial growth factor receptor), or ANG2R-Fc-region fusion polypeptides (ANG2R=human angiopoietin 2 receptor).

[0326] Fc-region fusion polypeptides may comprise e.g. a variant (human) IgG class Fc-region as reported herein and an antibody fragment that binds to a target including, such as, for example, an antibody Fab fragment, scFvs (see e.g. Nat. Biotechnol. 23 (2005) 1126-1136), or domain antibodies (dAbs) (see e.g. WO 2004/058821, WO 2003/002609). [0327] Fc-region fusion polypeptides may comprise e.g. a variant (human) IgG class Fc-region as reported herein and a receptor ligand (either naturally occurring or artificial).

[0328] Antibodies, e.g. full length antibodies or Cross-Mabs, can comprise a variant (human) human IgG class Fc-region as reported herein.

B. Ocular Vascular Diseases

[0329] Ocular vascular diseases are any pathological condition characterized by altered or unregulated proliferation and invasion of new blood vessels into the structures of ocular tissues such as the retina or cornea.

[0330] 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 (Sjogren's disease) and other ophthalmic diseases wherein the eye disease or disorder is associated with ocular neovascularization, vascular leakage, and/or retinal edema.

[0331] The antibody comprising the dimeric polypeptide as reported herein is useful in the prevention and treatment of wet AMD, dry AMD, CME, DME, NPDR, PDR, blepharitis, dry eye and uveitis, in one preferred embodiment wet AMD, dry AMD, blepharitis, and dry eye, also in one preferred embodiment CME, DME, NPDR and PDR, also in one preferred embodiment blepharitis, and dry eye, in particular wet AMD and dry AMD, and also particularly wet AMD.

[0332] In some embodiments, the ocular vascular 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.

[0333] 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, Sjogren's disease, 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, Wegener's sarcoidosis, Scleritis, Steven's Johnson disease, periphigoid radial keratotomy, and corneal graph rejection.

[0334] Diseases associated with retinal/choroidal neovascularization include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Paget's 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), Eale's disease, Bechet's disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargart's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications.

[0335] 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.

[0336] 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.

[0337] 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).

[0338] 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.

[0339] 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.

[0340] 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.

[0341] 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.

C. Antibody Purification with a *Staphylococcus*Protein a Affinity Chromatography Column

[0342] In one aspect, a dimeric polypeptide comprising

[0343] a first polypeptide and a second polypeptide each comprising in N-terminal to C-terminal direction at least a portion of an immunoglobulin hinge region, which comprises one or more cysteine residues, an immunoglobulin CH2-domain and an immunoglobulin CH3-domain,

[0344] wherein

[0345] i) the first and the second polypeptide comprise the mutations H310A, H433A and Y436A, or

[0346] ii) the first and the second polypeptide comprise the mutations L251D, L314D and L432D, or

[0347] iii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or

[0348] iv) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D

is provided.

[0349] These dimeric polypeptides have due to the mutations the properties of not binding to human FcRn whereas the binding to Staphylococcal protein A is maintained.

[0350] Thus, these antibodies can be purified, i.e. separated from unwanted by-products by using conventional protein A affinity materials, such as MabSelectSure. It is not required to use highly sophisticated but species limited affinity materials, such as e.g. KappaSelect, which is only useable with antibodies comprising a light chain of the kappa subclass. Additionally it is not required to adopt the purification method if a modification/exchange of the light chain subclass is made (see FIGS. 11 and 12, respectively).

[0351] One aspect as reported herein is a method for

[0351] One aspect as reported herein is a method for producing a dimeric polypeptide as reported herein comprising the following steps:

[0352] a) cultivating a mammalian cell comprising one or more nucleic acids encoding a dimeric polypeptide as reported herein,

[0353] b) recovering the dimeric polypeptide from the cultivation medium, and

[0354] c) purifying the dimeric polypeptide with a protein A affinity chromatography and thereby producing the dimeric polypeptide.

[0355] One aspect as reported herein is the use of the mutations H310A, H433A and Y436A for separating heterodimeric polypeptides from homodimeric polypeptides.

[0356] One aspect as reported herein is the use of the mutations L251D, L314D and L432D for separating heterodimeric polypeptides from homodimeric polypeptides.

[0357] One aspect as reported herein is the use of the mutations I253A, H310A and H435A in a first polypeptide in combination with the mutations H310A, H433A and Y436A in a second polypeptide for separating heterodimeric polypeptides comprising the first and the second polypeptide from homodimeric polypeptides.

[0358] One aspect as reported herein is the use of the mutations I253A, H310A and H435A in a first polypeptide in combination with the mutations L251D, L314D and L432D in a second polypeptide for separating heterodimeric polypeptides comprising the first and the second polypeptide from homodimeric polypeptides.

[0359] In one embodiment of the previous three aspects the first polypeptide further comprises the mutations Y349C, T366S, L368A and Y407V and the second polypeptide further comprises the mutations S354C and T366W.

[0360] In one embodiment of the previous three aspects the first polypeptide further comprises the mutations S354C, T366S, L368A and Y407V and the second polypeptide further comprises the mutations Y349C and T366W.

[0361] One aspect as reported herein is the use of the mutation Y436A for increasing the binding of a dimeric Fc-region polypeptide to Staphylococcal protein A.

[0362] It has been found that by introducing the mutation Y436A the binding of an Fc-region to Staphylococcal protein A (SPA) can be increased. This is advantageous e.g. if additional mutations are introduced that reduce the binding to SPA, such as e.g. I253A and H310A or H310A and H435A (see FIG. 15).

[0363] One aspect as reported herein is a dimeric polypeptide comprising

[0364] a first polypeptide and a second polypeptide each comprising in N-terminal to C-terminal direction at least a portion of an immunoglobulin hinge region, which comprises one or more cysteine residues, an immunoglobulin CH2-domain and an immunoglobulin CH3-domain.

[0365] wherein the first, the second or the first and the second polypeptide comprise the mutation Y436A (numbering according to the Kabat EU index numbering system).

[0366] In one embodiment the first and the second polypeptide comprise the mutation Y436A.

[0367] One aspect as reported herein is a bispecific antibody providing ease of isolation/purification comprising immunoglobulin heavy chain Fc-regions that are differentially modified, wherein at least one of the modifications results in i) a differential affinity of the bispecific antibody for protein A and ii) a differential affinity of the bispecific antibody for the human FcRn, and the bispecific antibody is isolable from a disrupted cell, from medium, or from a mixture of antibodies based on its affinity for protein A.

[0368] In one embodiment the bispecific antibody elutes at a pH value above pH 4.0.

[0369] In one embodiment the bispecific antibody is isolated using a protein A affinity chromatography and a pH gradient or pH step, wherein the pH gradient or pH step includes the addition of a salt. In a specific embodiment, the salt is present at a concentration of about 0.5 molar to about 1 molar. In one embodiment, the salt is selected from the group consisting of lithium, sodium, and potassium salts of acetate; sodium and potassium bicarbonates; lithium, sodium, and potassium carbonates; lithium, sodium, potassium, and magnesium chlorides; sodium and potassium fluorides; sodium, potassium, and calcium nitrates; sodium and potassium phosphates; and calcium and magnesium sulfates. In one embodiment the salt is a halide salt of an alkaline metal or alkaline earth metal. In one preferred embodiment the salt is sodium chloride.

[0370] In one aspect the dimeric polypeptide comprises a first polypeptide that is modified as reported herein and a second polypeptide that is not modified regarding protein A and FcRn binding, so as to form a heterodimeric polypeptide, wherein the differential modification results in the dimeric polypeptide eluting from a protein A affinity material at 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.3, or 1.4 pH unit(s) higher than a corresponding dimeric polypeptide that lacks the differential modification. In one embodiment, the differentially modified dimeric polypeptide elutes at a pH of 4 or higher, whereas the unmodified dimeric polypeptide elutes at a pH of 3.5 or lower. In one embodiment, the differentially modified dimeric polypeptide elutes at a pH of about 4, whereas the unmodified dimeric polypeptide elutes at a pH of about 2.8-3.5, 2.8-3.2, or 2.8-3. In these embodiments, "unmodified" refers to lack of the modification H310A, H433A and Y436A (Kabat EU index numbering system) in both of the polypeptides.

[0371] For chromatographic runs the addition of 0.5 molar to 1 molar salt (e.g. NaCl) may improve the separation of homodimeric polypeptide and heterodimeric polypeptide, especially if derived from the human IgG1 subclass. The addition of salt to the elution solution increasing the pH

value can broaden the pH range for elution such that e.g. a pH step gradient could successfully separate the two species. [0372] Accordingly, in one embodiment a method for separating a bispecific antibody comprising a heterodimeric IgG Fc-region with one chain comprising mutations as reported herein, comprises a step of employing a pH gradient in the presence of a salt. In one embodiment, the salt is present at a concentration sufficient to maximize the pH difference between elution from a protein A chromatography material of an IgG Fc-region homodimer and an IgG Fcregion heterodimer. In one embodiment the salt is present at a concentration of about 0.5 molar to about 1 molar. In one embodiment the salt is a salt of an alkaline metal or an alkaline earth metal and a halogen. In one embodiment the salt is a chloride salt of an alkaline metal or an alkaline earth metal, such as e.g. NaCl, KCl, LiCl, CaCl2, or MgCl2. In one embodiment the pH gradient is from about pH 4 to about pH 5. In one embodiment the gradient is a linear gradient. In one embodiment, the pH gradient is a step gradient. In one embodiment the method comprises applying to an equilibrated protein A affinity column a solution of about pH 4. In one embodiment the bispecific antibody comprising the heterodimeric IgG Fc-region with respect to the modifications as reported herein elutes from the protein A affinity chromatography material in one or more fractions substantially free of non-heterodimeric bispecific antibody.

[0373] The dimeric polypeptide as reported herein is produced by recombinant means. Thus, one aspect of the current invention is a nucleic acid encoding the dimeric polypeptide as reported herein and a further aspect is a cell comprising the nucleic acid encoding the dimeric polypeptide as reported herein. 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 dimeric polypeptide and usually purification to a pharmaceutically acceptable purity. For the expression of the dimeric polypeptides as aforementioned in a host cell, nucleic acids encoding the respective first and second polypeptides 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 dimeric polypeptide is recovered from the cells (cultivation supernatant or cells after lysis).

[0374] 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.

[0375] Accordingly one aspect as reported herein is a method for the production of a dimeric polypeptide as reported herein, comprising the steps of

[0376] a) transforming a host cell with one or more vectors comprising nucleic acid molecules encoding a dimeric polypeptide as reported herein,

[0377] b) culturing the host cell under conditions that allow synthesis of the dimeric polypeptide, and

[0378] c) recovering the dimeric polypeptide from the culture and thereby producing the dimeric polypeptide.

[0379] In one embodiment the recovering step under c) includes the use of an immunoglobulin Fc-region specific

capture reagent. In one embodiment this Fc-region specific capture reagent is used in a bind-and-elute-mode). Examples of such Fc-region specific capture reagents are e.g. *Staphylococcus* protein A-based affinity chromatography columns, 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 dimeric polypeptide, i.e. its Fc-region. The ligands are attached to the matrix via a long hydrophilic spacer arm to make it easily available for binding to the target molecule.

[0380] The dimeric polypeptides as reported herein 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. B-cells or hybridoma cells can serve as a source of DNA and RNA encoding the dimeric polypeptide. DNA and RNA encoding the monoclonal antibodies are readily isolated and sequenced using conventional procedures. 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 dimeric polypeptides, to obtain the synthesis of recombinant monoclonal dimeric polypeptides in the host cells

[0381] 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).

[0382] One aspect of the invention is a pharmaceutical formulation comprising a dimeric polypeptide or an antibody as reported herein. Another aspect of the invention is the use of a dimeric polypeptide or an antibody as reported herein for the manufacture of a pharmaceutical formulation. A further aspect of the invention is a method for the manufacture of a pharmaceutical formulation comprising a dimeric polypeptide or an antibody as reported herein. In another aspect, the present invention provides a formulation, e.g. a pharmaceutical formulation, containing a dimeric polypeptide or an antibody as reported herein, formulated together with a pharmaceutical carrier.

[0383] A formulation as reported herein 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.

[0384] 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 it's 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 it's not limited to eye drops to the cornea.

[0385] In one embodiment the dimeric polypeptide as reported herein or the pharmaceutical formulation as reported herein 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) 3266-3276; Russelakis-Carneiro et al., Neuropathol. Appl. Neurobiol. 25 (1999) 196-206; and Wray et al., Arch. Neurol. 33 (1976) 183-185.

[0386] In some embodiments, therapeutic kits of the invention can contain one or more doses of a dimeric polypeptide as reported herein present in a pharmaceutical formulation as described herein, a suitable device for intravitreal idjection of the pharmaceutical formulation, and an instruction detailing suitable subjects and protocols for carrying out the injection. In these embodiments, the formulations 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) 3266-3276; Russelakis-Carneiro et al., Neuropathol. Appl. Neurobiol. 25 (1999) 196-206; and Wray et al., Arch. Neurol. 33 (1976) 183-185).

[0387] The formulation 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 formulations. 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.

[0388] Regardless of the route of administration selected, the compounds as reported herein, which may be used in a suitable hydrated form, and/or the pharmaceutical formulations as reported herein, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

[0389] Actual dosage levels of the active ingredients in the pharmaceutical formulation as reported herein 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.

[0390] The formulation must be sterile and fluid to the extent that the formulation is deliverable by syringe. In addition to water, the carrier in one preferred embodiment is an isotonic buffered saline solution.

[0391] 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.

[0392] The formulation 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., a dimeric polypeptide as reported herein. The microparticles comprising a dimeric polypeptide as reported herein 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.

[0393] Another aspect of the invention is a dimeric polypeptide or an antibody as reported herein for use in the treatment of ocular vascular diseases.

[0394] One embodiment of the invention is a dimeric polypeptide or an antibody as reported herein for use in the treatment of ocular vascular diseases.

[0395] Another aspect of the invention is the pharmaceutical formulation for use in the treatment of ocular vascular diseases.

[0396] Another aspect of the invention is the use of a dimeric polypeptide or an antibody as reported herein for the manufacture of a medicament for the treatment of ocular vascular disease.

[0397] Another aspect of the invention is method of treatment of patient suffering from ocular vascular diseases by administering a dimeric polypeptide or an antibody as reported herein to a patient in the need of such treatment.

[0398] It is herewith expressly stated that the term "comprising" as used herein comprises the term "consisting of".

Thus, all aspects and embodiments that contain the term "comprising" are likewise disclosed with the term "consisting of".

D. Modifications

[0399] In a further aspect, a dimeric polypeptide according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-6 below:

1. Antibody Affinity

[0400] In one embodiment, Kd is measured using a BIA-CORE® surface plasmon resonance assay. For example, an assay using a BIACORE®-2000 or a BIACORE®-3000 (GE Healthcare Inc., Piscataway, N.J.) is performed at 25° C. with immobilized binding partner CM5 chips at ~10 response units (RU). In one embodiment, carboxymethylated dextran biosensor chips (CM5, GE Healthcare Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Binding partner is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/mL (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled binding partner. Following the injection of the binding partner, 1 M ethanolamine is injected to block non-reacted groups. For kinetics measurements, two-fold serial dilutions of the dimeric polypeptide containing fusion polypeptide or antibody (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25° C. at a flow rate of approximately 25 $\mu L/min.$ Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on} (see, e.g., Chen, Y. et al., J. Mol. Biol. 293 (1999) 865-881). If the on-rate exceeds 10^6 $M^{-1}s^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation=295 nm; emission=340 nm, 16 nm band-pass) at 25° C. of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCOTM spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Chimeric and Humanized Antibodies

[0401] In certain embodiments, a dimeric polypeptide as reported herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Pat. No. 4,816,567; and Morrison, S. L., et al., Proc. Natl. Acad. Sci. USA 81 (1984) 6851-6855). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0402] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0403] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro, J. C. and Fransson, J., Front. Biosci. 13 (2008) 1619-1633, and are further described, e.g., in Riechmann, I., et al., Nature 332 (1988) 323-329; Queen, C., et al., Proc. Natl. Acad. Sci. USA 86 (1989) 10029-10033; U.S. Pat. No. 5,821,337, U.S. Pat. No. 7,527,791, U.S. Pat. No. 6,982,321, and U.S. Pat. No. 7,087,409; Kashmiri, S. V., et al., Methods 36 (2005) 25-34 (describing specificity determining region (SDR) grafting); Padlan, E. A., Mol. Immunol. 28 (1991) 489-498 (describing "resurfacing"); Dall'Acqua, W. F. et al., Methods 36 (2005) 43-60 (describing "FR shuffling"); Osbourn, J. et al., Methods 36 (2005) 61-68; and Klimka, A. et al., Br. J. Cancer 83 (2000) 252-260 (describing the "guided selection" approach to FR shuffling).

[0404] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims, M. J., et al., J. Immunol. 151 (1993) 2296-2308; framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter, P., et al., Proc. Natl. Acad. Sci. USA 89 (1992) 4285-4289; and Presta, L. G., et al., J. Immunol. 151 (1993) 2623-2632); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro, J. C. and Fransson, J., Front. Biosci. 13 (2008) 1619-1633); and framework regions derived from screening FR libraries (see, e.g., Baca, M. et al., J. Biol. Chem. 272 (1997) 10678-10684 and Rosok, M. J. et al., J. Biol. Chem. 271 (19969 22611-22618).

3. Human Antibodies

[0405] In certain embodiments, a dimeric polypeptide as reported herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk, M. A. and van de Winkel, J. G., Curr. Opin. Pharmacol. 5 (2001) 368-374 and Lonberg, N., Curr. Opin. Immunol. 20 (2008) 450-459.

[0406] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For

review of methods for obtaining human antibodies from transgenic animals, see Lonberg, N., Nat. Biotech. 23 (2005) 1117-1125. See also, e.g., U.S. Pat. No. 6,075,181 and U.S. Pat. No. 6,150,584 describing XENOMOUSE™ technology; U.S. Pat. No. 5,770,429 describing HuMab® technology; U.S. Pat. No. 7,041,870 describing K-M MOUSE® technology, and US 2007/0061900, describing Veloci-Mouse® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[0407] Human antibodies can also be made by hybridomabased methods.

[0408] Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor, D., J. Immunol. 133 (1984) 3001-3005; Brodeur, B. R., et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York (1987), pp. 51-63; and Boerner, P., et al., J. Immunol. 147 (1991) 86-95). Human antibodies generated via human B-cell hybridoma technology are also described in Li, J., et al., Proc. Natl. Acad. Sci. USA 103 (2006) 3557-3562. Additional methods include those described, for example, in U.S. Pat. No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, J., Xiandai Mianyixue 26 (2006) 265-268 (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers, H. P. and Brandlein, S., Histology and Histopathology 20 (2005) 927-937 and Vollmers, H. P. and Brandlein, S., Methods and Findings in Experimental and Clinical Pharmacology 27 (2005) 185-191.

[0409] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

4. Library-Derived Antibodies

[0410] In certain embodiments a dimeric polypeptide as reported herein is a library-derived antibody. Library-derived antibodies may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom, H. R. et al., Methods in Molecular Biology 178 (2001) 1-37 and further described, e.g., in the McCafferty, J. et al., Nature 348 (1990) 552-554; Clackson, T. et al., Nature 352 (1991) 624-628; Marks, J. D. et al., J. Mol. Biol. 222 (1992) 581-597; Marks, J. D. and Bradbury, A., Methods in Molecular Biology 248 (2003) 161-175; Sidhu, S. S. et al., J. Mol. Biol. 338 (2004) 299-310; Lee, C. V. et al., J. Mol. Biol. 340 (2004) 1073-1093; Fellouse, F. A., Proc. Natl. Acad. Sci. USA 101 (2004) 12467-12472; and Lee, C. V. et al., J. Immunol. Methods 284 (2004) 119-132.

[0411] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter, G., et al., Ann. Rev. Immunol. 12 (1994) 433-455. Phage typically display antibody fragments, either

as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also selfantigens without any immunization as described by Griffiths, A. D., et al., EMBO J. 12 (1993) 725-734. Finally, naive libraries can also be made synthetically by cloning non-rearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom, H. R. and Winter, G., J. Mol. Biol. 227 (1992) 381-388. Patent publications describing human antibody phage libraries include, for example: U.S. Pat. No. 5,750,373, and US 2005/ 0079574, US 2005/0119455, US 2005/0266000, US 2007/ 0117126, US 2007/0160598, US 2007/0237764, US 2007/ 0292936, and US 2009/0002360.

[0412] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

5. Multispecific Antibodies

[0413] In certain embodiments, a dimeric polypeptide as reported herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for a first antigen and the other is for a different second antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of the same antigen. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express at least one of the antigens. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0414] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein, C. and Cuello, A. C., Nature 305 (1983) 537-540, WO 93/08829, and Traunecker, A., et al., EMBO J. 10 (1991) 3655-3659), and "knob-inhole" engineering (see, e.g., U.S. Pat. No. 5,731,168). Multispecific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004); cross-linking two or more antibodies or fragments (see, e.g., U.S. Pat. No. 4,676,980, and Brennan, M. et al., Science 229 (1985) 81-83); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny, S. A., et al., J. Immunol. 148 (1992) 1547-1553; using "diabody" technology for making bispecific antibody fragments (see, e.g., Holliger, P. et al., Proc. Natl. Acad. Sci. USA 90 (1993) 6444-6448); and using single-chain Fv (scFv) dimers (see, e.g. Gruber, M et al., J. Immunol. 152 (1994) 5368-5374); and preparing trispecific antibodies as described, e.g., in Tutt, A. et al., J. Immunol. 147 (1991) 60-69).

[0415] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576).

[0416] The antibody or fragment herein also includes a "Dual Acting Fab" or "DAF" (see, US 2008/0069820, for example).

[0417] The antibody or fragment herein also includes multispecific antibodies described in WO 2009/080251, WO 2009/080252, WO 2009/080253, WO 2009/080254, WO 2010/112193, WO 2010/115589, WO 2010/136172, WO 2010/145792, and WO 2010/145793.

6. Antibody Variants

[0418] In certain embodiments, a dimeric polypeptide as reported herein is an antibody. In further embodiment amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

a) Substitution, Insertion, and Deletion Variants

[0419] In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in the Table below under the heading of "preferred substitutions". More substantial changes are provided in the following Table under the heading of "exemplary substitutions", and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe;	Leu
	Norleucine	
Leu (L)	Norleucine; Ile; Val; Met;	Ile
	Ala; Phe	
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala;	Leu
	Norleucine	

[0420] Amino acids may be grouped according to common side-chain properties:

[0421] (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile:

[0422] (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

[0423] (3) acidic: Asp, Glu;

[0424] (4) basic: His, Lys, Arg;

[0425] (5) residues that influence chain orientation: Gly, Pro:

[0426] (6) aromatic: Trp, Tyr, Phe.

[0427] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0428] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0429] Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, P. S., Methods Mol. Biol. 207 (2008) 179-196), and/or residues that contact antigen, with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom, H. R. et al. in Methods in Molecular Biology 178 (2002) 1-37. In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0430] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may, for example, be outside of antigen contacting residues in the HVRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0431] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis

is called "alanine scanning mutagenesis" as described by Cunningham, B. C. and Wells, J. A., Science 244 (1989) 1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen can be used. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0432] Amino acid sequence insertions include aminoand/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation Variants

[0433] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0434] Where the antibody comprises an Fc-region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc-region. See, e.g., Wright, A. and Morrison, S. L., TIBTECH 15 (1997) 26-32. The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0435] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc-region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc-region (EU numbering of Fc-region residues); however, Asn297 may also be located about ±3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US 2003/0157108; US 2004/0093621. Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/ 61739; WO 2001/29246; US 2003/0115614; US 2002/ 0164328; US 2004/0093621; US 2004/0132140; US 2004/ 0110704; US 2004/0110282; US 2004/0109865; WO 2003/ 085119; WO 2003/084570; WO 2005/035586; WO 2005/ 035778; WO 2005/053742; WO 2002/031140; Okazaki, A. et al., J. Mol. Biol. 336 (2004) 1239-1249; Yamane-Ohnuki, N. et al., Biotech. Bioeng. 87 (2004) 614-622. Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka, J., et al., Arch. Biochem. Biophys. 249 (1986) 533-545; US 2003/0157108; and WO 2004/056312, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki, N., et al., Biotech. Bioeng. 87 (2004) 614-622; Kanda, Y., et al., Biotechnol. Bioeng. 94 (2006) 680-688; and WO 2003/085107).

[0436] Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc-region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878; U.S. Pat. No. 6,602,684; and US 2005/0123546. Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc-region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087; WO 1998/58964; and WO 1999/22764.

c) Fc-Region Variants

[0437] In certain embodiments, one or more further amino acid modifications may be introduced into a dimeric polypeptide as reported herein, thereby generating an Fc-region variant. The Fc-region variant may comprise a human Fcregion sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc-region) comprising an amino acid modification (e.g. a substitution/mutation) at one or more amino acid positions. [0438] In certain embodiments, the invention contemplates a dimeric polypeptide that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the dimeric polypeptide in vivo is important yet certain effector functions (such as CDC and ADCC) are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the dimeric polypeptide antibody lacks FcyR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcyRIII only, whereas monocytes express FcyRI, FcyRII and FcyRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch, J. V. and Kinet, J. P., Annu. Rev. Immunol. 9 (1991) 457-492. Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest are described in U.S. Pat. No. 5,500,362 (see, e.g. Hellstrom, I. et al., Proc. Natl. Acad. Sci. USA 83 (1986) 7059-7063; and Hellstrom, I. et al., Proc. Natl. Acad. Sci. USA 82 (1985) 1499-1502); U.S. Pat. No. 5,821,337 (see Bruggemann, M. et al., J. Exp. Med. 166 (1987) 1351-1361). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes, R. et al., Proc. Natl. Acad. Sci. USA 95 (1998) 652-656. Clq binding assays may also be carried out to confirm that the dimeric polypeptide is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro, H. et al., J. Immunol. Methods 202 (1996) 163-171; Cragg, M. S. et al., Blood 101 (2003) 1045-1052; and Cragg, M. S. and M. J. Glermie, Blood 103 (2004) 2738-2743). FcRn binding and in vivo clearance/half-life determinations can also be performed using methods known in the art (see, e.g., Petkova, S. B. et al., Int. Immunol. 18 (2006) 1759-1769).

[0439] Dimeric polypeptides with reduced effector function include those with substitution of one or more of Fc-region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc-region variants include Fc-regions with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc-region mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

[0440] Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Pat. No. 6,737,056; WO 2004/056312, and Shields, R. L. et al., J. Biol. Chem. 276 (2001) 6591-6604)

[0441] In certain embodiments, a dimeric polypeptide variant comprises an Fc-region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc-region (EU numbering of residues).

[0442] In some embodiments, alterations are made in the Fc-region that result in altered (i.e., either improved or diminished) Clq binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie, E. E. et al., J. Immunol. 164 (2000) 4178-4184.

[0443] Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer, R. L. et al., J. Immunol. 117 (1976) 587-593, and Kim, J. K. et al., J. Immunol. 24 (1994) 2429-2434), are described in US 2005/0014934. Those antibodies comprise an Fc-region with one or more substitutions therein which improve binding of the Fc-region to FcRn. Such Fc-region variants include those with substitutions at one or more of Fc-region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc-region residue 434 (U.S. Pat. No. 7,371,826).

[0444] See also Duncan, A. R. and Winter, G., Nature 322 (1988) 738-740; U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO 94/29351 concerning other examples of Fc-region variants.

d) Cysteine Engineered Antibody Variants

[0445] In certain embodiments, it may be desirable to create cysteine engineered dimeric polypeptides, e.g., in analogy to "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the dimeric polypeptide. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the dimeric polypeptide and may be used to conjugate the dimeric polypeptide to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc-region. Cysteine engineered dimeric polypeptides may be generated as described, e.g., in U.S. Pat. No. 7,521,541.

e) Derivatives

[0446] In certain embodiments, a dimeric polypeptide as reported herein may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the dimeric polypeptide include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or nonbranched. The number of polymers attached to the dimeric polypeptide may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the dimeric polypeptide to be improved, whether the dimeric polypeptide derivative will be used in a therapy under defined conditions, etc.

[0447] In another embodiment, conjugates of a dimeric polypeptide as reported herein and non-proteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the non-proteinaceous moiety is a carbon nanotube (Kam, N. W. et al., Proc. Natl. Acad. Sci. USA 102 (2005) 11600-11605). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the non-proteinaceous moiety to a temperature at which cells proximal to the dimeric polypeptide-non-proteinaceous moiety are killed.

f) Heterodimerization

[0448] There exist several approaches for CH3-modifications to enforce the heterodimerization, which are well described e.g. in WO 96/27011, WO 98/050431, EP 1870459, WO 2007/110205, WO 2007/147901, WO 2009/ 089004, WO 2010/129304, WO 2011/90754, WO 2011/ 143545, WO 2012058768, WO 2013157954, WO 2013096291. Typically in all such approaches the first CH3 domain and the second CH3 domains are both engineered in a complementary manner so that each CH3 domain (or the heavy chain comprising it) cannot longer homodimerize with itself but is forced to heterodimerize with the complementary engineered other CH3 domain (so that the first and second CH3 domain heterodimerize and no homodimers between the two first or the two second CH3 domains are formed). These different approaches for improved heavy chain heterodimerization are contemplated as different alternatives in combination with the heavy-light chain modifications (VH and VL exchange/replacement in one binding arm and the introduction of substitutions of charged amino acids with opposite charges in the CH1/CL interface) in the multispecific antibodies according to the invention which reduce light chain mispairing an Bence-Jones type side products.

[0449] In one preferred embodiment of the invention (in case the multispecific antibody comprises CH3 domains in the heavy chains) the CH3 domains of said multispecific antibody according to the invention can be 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; WO 98/050431. In this method the interaction surfaces of the two CH3 domains are altered to increase the heterodimerization 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 further 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.

[0450] Thus in one embodiment of the invention said multispecific antibody (comprises a CH3 domain in each heavy chain and) is further characterized in that

- [0451] the first CH3 domain of the first heavy chain of the antibody under a) and the second CH3 domain of the second heavy chain of the antibody under b) each meet at an interface which comprises an original interface between the antibody CH3 domains.
 - [0452] wherein said interface is altered to promote the formation of the multispecific antibody, wherein the alteration is characterized in that:
 - [0453] i) the CH3 domain of one heavy chain is altered,
 - [0454] so that within the original interface of the CH3 domain of one heavy chain that meets the original interface of the CH3 domain of the other heavy chain within the multispecific antibody,
 - [0455] 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
 - [0456] ii) the CH3 domain of the other heavy chain is altered,

[0457] so that within the original interface of the second CH3 domain that meets the original interface of the first CH3 domain within the multispecific antibody

[0458] 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.

[0459] 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).

[0460] 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).

[0461] 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.

[0462] In one preferred embodiment, said multispecific antibody comprises a amino acid T366W mutation in the first CH3 domain of the "knobs chain" and amino acid T366S, L368A, Y407V mutations in the second 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 an amino acid Y349C mutation into the CH3 domain of the "hole chain" and an amino acid E356C mutation or an amino acid S354C mutation into the CH3 domain of the "knobs chain".

[0463] In one preferred embodiment, said multispecific antibody (which comprises a CH3 domain in each heavy chain) comprises amino acid S354C, T366W mutations in one of the two CH3 domains and amino acid Y349C, T366S, L368A, Y407V mutations in the other of the two CH3 domains (the additional amino acid S354C mutation in one CH3 domain and the additional amino acid Y349C mutation in the other CH3 domain forming an interchain disulfide bridge) (numbering according to Kabat).

[0464] Other techniques for CH3-modifications to enforcing the heterodimerization are contemplated as alternatives of the invention and described e.g. in WO 96/27011, WO 98/050431, EP 1870459, WO 2007/110205, WO 2007/147901, WO 2009/089004, WO 2010/129304, WO 2011/90754, WO 2011/143545, WO 2012/058768, WO 2013/157954, WO 2013/096291.

[0465] In one embodiment the heterodimerization approach described in EP 1 870 459A1, can be used alternatively. This approach is based on the by the introduction of substitutions/mutations of charged amino acids with the opposite charge at specific amino acid positions of the in the CH3/CH3 domain interface between both heavy chains. One preferred embodiment for said multispecific antibody are amino acid R409D; K370E mutations in the first CH3 domain of the (of the multispecific antibody) and amino acid D399K; E357K mutations in the seconds CH3 domain of the multispecific antibody (numbering according to Kabat).

[0466] In another embodiment said multispecific antibody comprises a amino acid T366W mutation in the CH3 domain of the "knobs chain" and amino acid T366S, L368A, Y407V mutations in the CH3 domain of the "hole chain" and

additionally amino acid R409D; K370E mutations in the CH3 domain of the "knobs chain" and amino acid D399K; E357K mutations in the CH3 domain of the "hole chain". [0467] In another embodiment said multispecific antibody comprises amino acid S354C, T366W mutations in one of the two CH3 domains and amino acid Y349C, T366S, L368A, Y407V mutations in the other of the two CH3 domains or said multispecific antibody comprises amino acid Y349C, T366W mutations in one of the two CH3 domains and amino acid S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains and additionally amino acid R409D; K370E mutations in the CH3 domain of the "knobs chain" and amino acid D399K; E357K mutations in the CH3 domain of the "hole chain".

[0468] In one embodiment the heterodimerization approach described in WO2013/157953 can be used alternatively. In one embodiment a first CH3 domain comprises amino acid T366K mutation and a second CH3 domain polypeptide comprises amino acid L351D mutation. In a further embodiment the first CH3 domain comprises further amino acid L351K mutation. In a further embodiment the second CH3 domain comprises further amino acid mutation selected from Y349E, Y349D and L368E (preferably L368E).

[0469] In one embodiment the heterodimerization approach described in WO2012/058768 can be used alternatively. In one embodiment a first CH3 domain comprises amino acid L351Y, Y407A mutations and a second CH3 domain comprises amino acid T366A, K409F mutations. In a further embodiment the second CH3 domain comprises a further amino acid mutation at position T411, D399, S400, F405, N390, or K392 e.g. selected from a) T411 N, T411 R, T411Q, T411 K, T411D, T411E or T411W, b) D399R, D399W, D399Y or D399K, c S400E, S400D, S400R, or S400K F405I, F405M, F405T, F405S, F405V or F405W N390R, N390K or N390D K392V, K392M, K392R, K392L, K392F or K392E. In a further embodiment a first CH3 domain comprises amino acid L351Y, Y407A mutations and a second CH3 domain comprises amino acid T366V, K409F mutations. In a further embodiment a first CH3 domain comprises amino acid Y407A mutations and a second CH3 domain comprises amino acid T366A, K409F mutations. In a further embodiment the second CH3 domain comprises a further amino acid K392E, T411E, D399R and S400R mutations.

[0470] In one embodiment the heterodimerization approach described in WO2011/143545 can be used alternatively e.g. with the amino acid modification at a position selected from the group consisting of 368 and 409.

[0471] In one embodiment the heterodimerization approach described in WO2011/090762 which also uses the knobs-into-holes technology described above can be used alternatively. In one embodiment a first CH3 domain comprises amino acid T366W mutations and a second CH3 domain comprises amino acid Y407A mutations. In one embodiment a first CH3 domain comprises amino acid T366Y mutations and a second CH3 domain comprises amino acid Y407T mutations.

[0472] In one embodiment the multispecific antibody is of IgG2 isotype and the heterodimerization approach described in WO2010/129304 can be used alternatively.

[0473] In one embodiment the heterodimerization approach described in WO2009/089004 can be used alternatively. In one embodiment a first CH3 domain comprises

amino acid substitution of K392 or N392 with a negative-charged amino acid (e.g. glutamic acid (E), or aspartic acid (D), preferably K392D or N392D) and a second CH3 domain comprises amino acid substitution of D399, E356, D356, or E357 with a positive-charged amino acid (e.g. Lysine (K) or arginine (R), preferably D399K, E356K, D356K, or E357K and more preferably D399K and E356K. In a further embodiment the first CH3 domain further comprises amino acid substitution of K409 or R409 with a negative-charged amino acid (e.g. glutamic acid (E), or aspartic acid (D), preferably K409D or R409D). In a further embodiment the first CH3 domain further or alternatively comprises amino acid substitution of K439 and/or K370 with a negative-charged amino acid (e.g. glutamic acid (E), or aspartic acid (D)).

[0474] In one embodiment the heterodimerization approach described in WO2007/147901 can be used alternatively. In one embodiment a first CH3 domain comprises amino acid K253E, D282K, and K322D mutations and a second CH3 domain comprises amino acid D239K, E240K, and K292D mutations.

[0475] In one embodiment the heterodimerization approach described in WO2007/110205 can be used alternatively.

E. Recombinant Methods and Compositions

[0476] Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Pat. No. 4,816,567. In one embodiment, isolated nucleic acid(s) encoding a dimeric polypeptide as reported herein is(are) provided. Such nucleic acid may encode an amino acid sequence comprising the first polypeptide and/or an amino acid sequence comprising the second polypeptide of the dimeric polypeptide. In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the first polypeptide of the dimeric polypeptide and an amino acid sequence comprising the second polypeptide of the dimeric polypeptide, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the first polypeptide of the dimeric polypeptide and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the second polypeptide of the dimeric polypeptide. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one embodiment, a method of making a dimeric polypeptide as reported herein is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the dimeric polypeptide, as provided above, under conditions suitable for expression of the dimeric polypeptide, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0477] For recombinant production of a dimeric polypeptide as reported herein, nucleic acid encoding a dimeric polypeptide, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifi-

cally to genes encoding the variant Fc-region polypeptide(s) and the heavy and light chains of the antibody).

[0478] Suitable host cells for cloning or expression of dimeric polypeptide-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, dimeric polypeptides may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Pat. No. 5,648,237, U.S. Pat. No. 5,789,199, and U.S. Pat. No. 5,840,523. (See also Charlton, K. A., In: Methods in Molecular Biology, Vol. 248, Lo, B. K. C. (ed.), Humana Press, Totowa, N.J. (2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.). After expression, the dimeric polypeptide may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0479] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for dimeric polypeptide-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized" resulting in the production of a dimeric polypeptide with a partially or fully human glycosylation pattern. See Gerngross, T. U., Nat. Biotech. 22 (2004) 1409-1414; and Li, H. et al., Nat. Biotech. 24 (2006) 210-215.

[0480] Suitable host cells for the expression of glycosylated a dimeric polypeptide are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

[0481] Plant cell cultures can also be utilized as hosts. See, e.g., U.S. Pat. No. 5,959,177, U.S. Pat. No. 6,040,498, U.S. Pat. No. 6,420,548, U.S. Pat. No. 7,125,978, and U.S. Pat. No. 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

[0482] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (HEK293 or 293 cells as described, e.g., in Graham, F. L., et al., J. Gen Virol. 36 (1977) 59-74); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, J. P., Biol. Reprod. 23 (1980) 243-252); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK); buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather, J. P., et al., Annals N.Y. Acad. Sci. 383 (1982) 44-68; MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR- CHO cells (Urlaub, G., et al., Proc. Natl. Acad. Sci. USA 77 (1980) 4216-4220); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki, P. and Wu, A. M., Methods in Molecular Biology, Vol. 248, Lo, B. K. C. (ed.), Humana Press, Totowa, N.J. (2004), pp. 255-268.

F. Combination Treatment

[0483] In certain embodiments the dimeric polypeptide as reported herein or pharmaceutical formulation as reported herein is administered alone (without an additional therapeutic agent) for the treatment of one or more ocular vascular diseases described herein.

[0484] In other embodiments the dimeric polypeptide antibody or pharmaceutical formulation as reported herein is administered in combination with one or more additional therapeutic agents or methods for the treatment of one or more vascular eye diseases described herein.

[0485] In other embodiments, the dimeric polypeptide or pharmaceutical formulation as reported herein is formulated in combination with one or more additional therapeutic agents and administered for the treatment of one or more vascular eye diseases described herein.

[0486] In certain embodiments, the combination treatments provided herein include that the dimeric polypeptide or pharmaceutical formulation as reported herein is administered sequentially with one or more additional therapeutic agents for the treatment of one or more ocular vascular diseases described herein.

[0487] The additional therapeutic agents include, but are not limited to, Tryptophanyl-tRNA synthetase (TrpRS), EyeOO1 (anti-VEGF PEGylated aptamer), squalamine, RETAANETM (anecortave acetate for depot suspension; Alcon, Inc.), Combretastatin A4 Prodrug (CA4P), MACU-GENTM, MIFEPREXTM (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-(1-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.

[0488] Other pharmaceutical therapies that can be used in combination with the dimeric polypeptide or pharmaceutical formulation as reported herein, including, but are not limited to, VISUDYNETM 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 plc.), 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, receptortargeted 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.

[0489] Any anti-angiogenic agent can be used in combination with the dimeric polypeptide or pharmaceutical formulation as reported herein, including, but not limited to, those listed by Carmeliet and Jain (Nature 407 (2000) 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, Deltalike 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 rapamycin, everolismus, temsirolismus, cyclospohne, anti-TNF agents, anti-complement agents, and non-steroidal anti-inflammatory 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® and Fidelin®), dehydroepiandrosterone sulfate, and pregnenolone sulfate. Any AMD (age-related macular degeneration) therapeutic agent can be used in combination with the dimeric polypeptide or pharmaceutical formulation as reported herein, including but not limited to verteporfin in combination with PDT, pegaptanib sodium, zinc, or an antioxidant(s), alone or in any combination.

G. Pharmaceutical Formulations

[0490] Pharmaceutical formulations of a dimeric polypeptide as reported herein are prepared by mixing such dimeric polypeptide having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed.) (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyl dimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as poly(vinylpyrrolidone); amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rhuPH20 (HYL-ENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rhuPH20, are described in US 2005/0260186 and US 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0491] Exemplary lyophilized antibody formulations are described in U.S. Pat. No. 6,267,958. Aqueous antibody formulations include those described in U.S. Pat. No. 6,171, 586 and WO 2006/044908, the latter formulations including a histidine-acetate buffer.

[0492] The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0493] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methyl methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules)

or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed.) (1980).

[0494] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

[0495] The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

H. Therapeutic Methods and Compositions

[0496] Any of the dimeric polypeptides as reported herein may be used in therapeutic methods.

[0497] In one aspect, a dimeric polypeptide as reported herein for use as a medicament is provided. In further aspects, a dimeric polypeptide for use in treating ocular vascular diseases is provided. In certain embodiments, a dimeric polypeptide for use in a method of treatment is provided. In certain embodiments, the invention provides a dimeric polypeptide for use in a method of treating an individual having an ocular vascular disease comprising administering to the individual an effective amount of the dimeric polypeptide as reported herein. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described above in section D. In further embodiments, the invention provides a dimeric polypeptide for use in inhibiting angiogenesis in the eye. In certain embodiments, the invention provides a dimeric polypeptide for use in a method of inhibiting angiogenesis in an individual comprising administering to the individual an effective of the dimeric polypeptide to inhibit angiogenesis. An "individual" according to any of the above embodiments is in one preferred embodiment a human.

[0498] In a further aspect, the invention provides for the use of a dimeric polypeptide in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of an ocular vascular disease. In a further embodiment, the medicament is for use in a method of treating an ocular vascular disease comprising administering to an individual having an ocular vascular disease an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described above. In a further embodiment, the medicament is for inhibiting angiogenesis. In a further embodiment, the medicament is for use in a method of inhibiting angiogenesis in an individual comprising administering to the individual an amount effective of the medicament to inhibit angiogenesis. An "individual" according to any of the above embodiments may be a human.

[0499] In a further aspect, the invention provides a method for treating a vascular eye disease. In one embodiment, the method comprises administering to an individual having such a vascular eye disease an effective amount of a dimeric polypeptide as reported herein. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An "individual" according to any of the above embodiments may be a human.

[0500] In a further aspect, the invention provides a method for inhibiting angiogenesis in the eye in an individual. In one embodiment, the method comprises administering to the individual an effective amount of a dimeric polypeptide as reported herein to inhibit angiogenesis. In one embodiment, an "individual" is a human.

[0501] In a further aspect, the invention provides pharmaceutical formulations comprising any of the dimeric polypeptides as reported herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the dimeric polypeptides as reported herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the dimeric polypeptides as reported herein and at least one additional therapeutic agent, e.g., as described below

[0502] Dimeric polypeptide as reported herein can be used either alone or in combination with other agents in a therapy. For instance, a dimeric polypeptide as reported herein may be co-administered with at least one additional therapeutic agent

[0503] A dimeric polypeptide as reported herein (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraveneral, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0504] Dimeric polypeptides as reported herein would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The dimeric polypeptide need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of dimeric polypeptide present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0505] For the prevention or treatment of disease, the appropriate dosage of a dimeric polypeptide as reported herein (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of dimeric polypeptide, the severity and course of the disease, whether the dimeric polypeptide is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the dimeric polypeptide, and the discretion of the attending physician. The dimeric polypeptide is suitably administered to the patient at one time or over a series of

treatments. Depending on the type and severity of the disease, about 1 μ g/kg to 15 mg/kg (e.g. 0.5 mg/kg-10 mg/kg) of dimeric polypeptide can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the dimeric polypeptide would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the dimeric polypeptide). An initial higher loading dose, followed by one or more lower doses may be administered. The progress of this therapy is easily monitored by conventional techniques and assays.

III. Articles of Manufacture

[0506] In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a dimeric polypeptide as reported herein. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a dimeric polypeptide as reported herein; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0507] It is understood that any of the above articles of manufacture may include an immunoconjugate as reported herein in place of or in addition to a dimeric polypeptide as reported herein.

IV. Specific Embodiments

[0508] 1. A dimeric polypeptide comprising

[0509] a first polypeptide and a second polypeptide each comprising in N-terminal to C-terminal direction at least a portion of an immunoglobulin hinge region, which comprises one or more cysteine residues, an immunoglobulin CH2-domain and an immunoglobulin CH3-domain,

[0510] wherein

- [0511] i) the first and the second polypeptide comprise the mutations H310A, H433A and Y436A, or
- [0512] ii) the first and the second polypeptide comprise the mutations L251D, L314D and L432D, or
- [0513] iii) the first and the second polypeptide comprise the mutations L251S, L314S and L432S, or
- [0514] iv) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
- [0515] v) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
- [0516] vi) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S.
- [0517] 2. The dimeric polypeptide according to item 1, characterized in that the dimeric polypeptide does not specifically bind to the human FcRn and does specifically bind to Staphylococcal protein A.
- [0518] 3. The dimeric polypeptide according to any one of items 1 to 2, characterized in that the dimeric polypeptide is a homodimeric polypeptide.
- [0519] 4. The dimeric polypeptide according to any one of items 1 to 2, characterized in that the dimeric polypeptide is a heterodimeric polypeptide.
- [0520] 5. The dimeric polypeptide according to any one of items 1 to 4, characterized in that i) the first polypeptide further comprises the mutations Y349C, T366S, L368A and Y407V and the second polypeptide comprises the mutations S354C and T366W, or ii) the first polypeptide further comprises the mutations S354C, T366S, L368A and Y407V and the second polypeptide comprises the mutations Y349C and T366W.
- [0521] 6. The dimeric polypeptide according to any one of items 1 to 5, characterized in that the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain are of the human IgG1 subclass.
- [0522] 7. The dimeric polypeptide according to any one of items 1 to 6, characterized in that the first polypeptide and the second polypeptide further comprise the mutations L234A and L235A.
- [0523] 8. The dimeric polypeptide according to any one of items 1 to 5, characterized in that the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain are of the human IgG2 subclass optionally with the mutations V234A, G237A, P238S, H268A, V309L, A330S and P331S.
- [0524] 9. The dimeric polypeptide according to any one of items 1 to 5, characterized in that the immunoglobulin

- hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain are of the human IgG4 subclass
- [0525] 10. The dimeric polypeptide according to any one of items 1 to 5 and 9, characterized in that the first polypeptide and the second polypeptide further comprise the mutations S228P and L235E.
- [0526] 11. The dimeric polypeptide according to any one of items 1 to 10, characterized in that the first polypeptide and the second polypeptide further comprise the mutation P329G.
- [0527] 12. The dimeric polypeptide according to any one of items 1 to 11, characterized in that the dimeric polypeptide is an Fc-region fusion polypeptide.
- [0528] 13. The dimeric polypeptide according to any one of items 1 to 11, characterized in that the dimeric polypeptide is an (full length) antibody.
- [0529] 14. The dimeric polypeptide according to any one of items 1 to 11 and 13, characterized in that the (full length) antibody is a monospecific antibody.
- [0530] 15. The dimeric polypeptide according to any one of items 1 to 11 and 13 to 14, characterized in that the monospecific antibody is a monovalent monospecific antibody.
- [0531] 16. The dimeric polypeptide according to any one of items 1 to 11 and 13 to 15, characterized in that the monospecific antibody is a bivalent monospecific antibody.
- [0532] 17. The dimeric polypeptide according to any one of items 1 to 11 and 13, characterized in that the (full length) antibody is a bispecific antibody.
- [0533] 18. The dimeric polypeptide according to any one of items 1 to 11 and 13 and 17, characterized in that the bispecific antibody is a bivalent bispecific antibody.
- [0534] 19. The dimeric polypeptide according to any one of items 1 to 11 and 13 and 17 to 18, characterized in that the bispecific antibody is a tetravalent bispecific antibody.
- [0535] 20. The dimeric polypeptide according to any one of items 1 to 11 and 13, characterized in that the (full length) antibody is a trispecific antibody.
- [0536] 21. The dimeric polypeptide according to any one of items 1 to 11 and 13 and 20, characterized in that the trispecific antibody is a trivalent trispecific antibody.
- [0537] 22. The dimeric polypeptide according to any one of items 1 to 11 and 13 and 20 to 21, characterized in that the trispecific antibody is a tetravalent trispecific antibody.
- [0538] 23. A dimeric polypeptide comprising
 - [0539] a first polypeptide and a second polypeptide each comprising in N-terminal to C-terminal direction at least a portion of an immunoglobulin hinge region, which comprises one or more cysteine residues, an immunoglobulin CH2-domain and an immunoglobulin CH3-domain,
 - [0540] wherein the first, the second or the first and the second polypeptide comprise the mutation Y436A (numbering according to the Kabat EU index numbering system).
- [0541] 24. The dimeric polypeptide according to item 23, characterized in that the first and the second polypeptide comprise the mutation Y436A.
- [0542] 25. The dimeric polypeptide according to any one of items 23 to 24, characterized in that the dimeric

- polypeptide does not specifically bind to the human FcRn and does specifically bind to Staphylococcal protein A.
- [0543] 26. The dimeric polypeptide according to any one of items 23 to 25, characterized in that the dimeric polypeptide is a homodimeric polypeptide.
- [0544] 27. The dimeric polypeptide according to any one of items 23 to 25, characterized in that the dimeric polypeptide is a heterodimeric polypeptide.
- [0545] 28. The dimeric polypeptide according to any one of items 23 to 27, characterized in that
 - [0546] a) the first polypeptide further comprises the mutations Y349C, T366S, L368A and Y407V and the second polypeptide comprises the mutations S354C and T366W,
 - [0547] or
 - [0548] the first polypeptide further comprises the mutations S354C, T366S, L368A and Y407V and the second polypeptide comprises the mutations Y349C and T366W, and/or
 - [0549] b) i) the first and the second polypeptide comprise the mutations H310A, H433A and Y436A, or
 - [0550] ii) the first and the second polypeptide comprise the mutations L251D, L314D and L432D, or
 - [0551] iii) the first and the second polypeptide comprise the mutations L251S, L314S and L432S, or
 - [0552] iv) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
 - [0553] v) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
 - [0554] vi) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S.
- [0555] 29. The dimeric polypeptide according to any one of items 23 to 28, characterized in that the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain are of the human IgG1 subclass.
- [0556] 30. The dimeric polypeptide according to any one of items 23 to 29, characterized in that the first polypeptide and the second polypeptide further comprise the mutations L234A and L235A.
- [0557] 31. The dimeric polypeptide according to any one of items 23 to 28, characterized in that the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain are of the human IgG2 subclass optionally with the mutations V234A, G237A, P238S, H268A, V309L, A330S and P331S.
- [0558] 32. The dimeric polypeptide according to any one of items 23 to 28, characterized in that the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain are of the human IgG4 subclass.
- [0559] 33. The dimeric polypeptide according to any one of items 23 to 28 and 32, characterized in that the first polypeptide and the second polypeptide further comprise the mutations S228P and L235E.

- [0560] 34. The dimeric polypeptide according to any one of items 23 to 33, characterized in that the first polypeptide and the second polypeptide further comprise the mutation P329G.
- [0561] 35. The dimeric polypeptide according to any one of items 23 to 34, characterized in that the dimeric polypeptide is an Fc-region fusion polypeptide.
- [0562] 36. The dimeric polypeptide according to any one of items 23 to 34, characterized in that the dimeric polypeptide is an (full length) antibody.
- [0563] 37. The dimeric polypeptide according to any one of items 23 to 34 and 36, characterized in that the (full length) antibody is a monospecific antibody.
- [0564] 38. The dimeric polypeptide according to any one of items 23 to 34 and 36 to 37, characterized in that the monospecific antibody is a monovalent monospecific antibody.
- [0565] 39. The dimeric polypeptide according to any one of items 23 to 34 and 36 to 38, characterized in that the monospecific antibody is a bivalent monospecific antibody.
- [0566] 40. The dimeric polypeptide according to any one of items 23 to 34 and 36, characterized in that the (full length) antibody is a bispecific antibody.
- [0567] 41. The dimeric polypeptide according to any one of items 23 to 34 and 36 and 40, characterized in that the bispecific antibody is a bivalent bispecific antibody.
- [0568] 42. The dimeric polypeptide according to any one of items 23 to 34 and 36 and 40 to 41, characterized in that the bispecific antibody is a tetravalent bispecific antibody.
- [0569] 43. The dimeric polypeptide according to any one of items 23 to 34 and 36, characterized in that the (full length) antibody is a trispecific antibody.
- [0570] 44. The dimeric polypeptide according to any one of items 23 to 34 and 36 and 43, characterized in that the trispecific antibody is a trivalent trispecific antibody.
- [0571] 45. The dimeric polypeptide according to any one of items 23 to 34 and 36 and 43 to 44, characterized in that the trispecific antibody is a tetravalent trispecific antibody.
- [0572] 46. A dimeric polypeptide comprising
 - [0573] a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1 and an immunoglobulin CH3-domain of the subclass IgG1,
 - [0574] a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1 and an immunoglobulin CH3-domain of the subclass IgG1,
 - [0575] a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and a light chain constant domain,
 - [0576] a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,
 - [0577] wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen,

- [0578] wherein the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a second antigen,
- [0579] wherein i) the first polypeptide comprises the mutations Y349C, T366S, L368A and Y407V and the second polypeptide comprises the mutations S354C and T366W, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A and Y407V and the second polypeptide comprises the mutations Y349C and T366W.
- [0580] wherein the first and the second polypeptide further comprise the mutations L234A, L235A and P329G, and
- [0581] wherein
 - [0582] i) the first and the second polypeptide comprise the mutations H310A, H433A and Y436A, or
 - [0583] ii) the first and the second polypeptide comprise the mutations L251D, L314D and L432D, or
 - [0584] iii) the first and the second polypeptide comprise the mutations L251S, L314S and L432S, or
 - [0585] iv) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
 - [0586] v) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
 - [0587] vi) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S.
- [0588] 47. A dimeric polypeptide comprising
 - [0589] a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin light chain constant domain, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1 and an immunoglobulin CH3-domain of the subclass IgG1,
 - [0590] a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1 and an immunoglobulin CH3-domain of the subclass IgG1,
 - [0591] a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and an immunoglobulin CH1-domain of the subclass IgG1,
 - [0592] a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,
 - [0593] wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen,
 - [0594] wherein the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a second antigen,
 - [0595] wherein i) the first polypeptide comprises the mutations Y349C, T366S, L368A and Y407V and the

- second polypeptide comprises the mutations S354C and T366W, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A and Y407V and the second polypeptide comprises the mutations Y349C and T366W,
- [0596] wherein the first and the second polypeptide further comprise the mutations L234A, L235A and P329G, and
- [0597] wherein
 - [0598] i) the first and the second polypeptide comprise the mutations H310A, H433A and Y436A, or
 - [0599] ii) the first and the second polypeptide comprise the mutations L251D, L314D and L432D, or
 - [0600] iii) the first and the second polypeptide comprise the mutations L251S, L314S and L432S, or
 - [0601] iv) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
 - [0602] v) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
 - [0603] vi) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S.
- [0604] 48. A dimeric polypeptide comprising
 - [0605] a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG4, an immunoglobulin hinge region of the subclass IgG4, an immunoglobulin CH2-domain of the subclass IgG4 and an immunoglobulin CH3-domain of the subclass IgG4,
 - [0606] a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG4, an immunoglobulin hinge region of the subclass IgG4, an immunoglobulin CH2-domain of the subclass IgG4 and an immunoglobulin CH3-domain of the subclass IgG4,
 - [0607] a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and a light chain constant domain,
 - [0608] a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,
 - [0609] wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen,
 - [0610] wherein the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a second antigen.
 - [0611] wherein i) the first polypeptide comprises the mutations Y349C, T366S, L368A and Y407V and the second polypeptide comprises the mutations S354C and T366W, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A and Y407V and the second polypeptide comprises the mutations Y349C and T366W,

[0612] wherein the first and the second polypeptide further comprise the mutations S228P, L235E and P329G, and

[0613] wherein

[0614] i) the first and the second polypeptide comprise the mutations H310A, H433A and Y436A, or

[0615] ii) the first and the second polypeptide comprise the mutations L251D, L314D and L432D, or

[0616] iii) the first and the second polypeptide comprise the mutations L251S, L314S and L432S, or

[0617] iv) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or

[0618] v) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or

[0619] vi) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S.

[0620] 49. A dimeric polypeptide comprising

[0621] a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin light chain constant domain, an immunoglobulin hinge region of the subclass IgG4, an immunoglobulin CH2-domain of the subclass IgG4 and an immunoglobulin CH3-domain of the subclass IgG4,

[0622] a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG4, an immunoglobulin hinge region of the subclass IgG4, an immunoglobulin CH2-domain of the subclass IgG4 and an immunoglobulin CH3-domain of the subclass IgG4,

[0623] a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and an immunoglobulin CH1-domain of the subclass IgG4.

[0624] a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,

[0625] wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen,

[0626] wherein the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a second antigen,

[0627] wherein i) the first polypeptide comprises the mutations Y349C, T366S, L368A and Y407V and the second polypeptide comprises the mutations S354C and T366W, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A and Y407V and the second polypeptide comprises the mutations Y349C and T366W,

[0628] wherein the first and the second polypeptide further comprise the mutations S228P, L235E and P329G, and

[0629] wherein

[0630] i) the first and the second polypeptide comprise the mutations H310A, H433A and Y436A, or

[0631] ii) the first and the second polypeptide comprise the mutations L251D, L314D and L432D, or

[0632] iii) the first and the second polypeptide comprise the mutations L251S, L314S and L432S, or

[0633] iv) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or

[0634] v) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or

[0635] vi) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S.

[0636] 50. A dimeric polypeptide comprising

[0637] a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1, an immunoglobulin CH3-domain of the subclass IgG1, a peptidic linker and a first scFv,

[0638] a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1, an immunoglobulin CH3-domain of the subclass IgG1, a peptidic linker and a second scFv,

[0639] a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and a light chain constant domain,

[0640] a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,

[0641] wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen, the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a first antigen, the first and the second scFv specifically bind to a second antigen,

[0642] wherein i) the first polypeptide comprises the mutations Y349C, T366S, L368A and Y407V and the second polypeptide comprises the mutations S354C and T366W, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A and Y407V and the second polypeptide comprises the mutations Y349C and T366W,

[0643] wherein the first and the second polypeptide further comprise the mutations L234A, L235A and P329G, and

[0644] wherein

[0645] i) the first and the second polypeptide comprise the mutations H310A, H433A and Y436A, or

[0646] ii) the first and the second polypeptide comprise the mutations L251D, L314D and L432D, or

[0647] iii) the first and the second polypeptide comprise the mutations L251S, L314S and L432S, or

- [0648] iv) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
- [0649] v) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
- [0650] vi) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S.
- [0651] 51. A dimeric polypeptide comprising
 - [0652] a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin light chain constant domain, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1, an immunoglobulin CH3-domain of the subclass IgG1, a peptidic linker and a first scFv,
 - [0653] a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1, an immunoglobulin CH3-domain of the subclass IgG1, a peptidic linker and a second scFv,
 - [0654] a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and an immunoglobulin CH1-domain of the subclass IgG1,
 - [0655] a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,
 - [0656] wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen, the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a first antigen, and the first and the second scFv specifically bind to a second antigen,
 - [0657] wherein i) the first polypeptide comprises the mutations Y349C, T366S, L368A and Y407V and the second polypeptide comprises the mutations S354C and T366W, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A and Y407V and the second polypeptide comprises the mutations Y349C and T366W.
 - [0658] wherein the first and the second polypeptide further comprise the mutations L234A, L235A and P329G, and
 - [0659] wherein
 - [0660] i) the first and the second polypeptide comprise the mutations H310A, H433A and Y436A, or
 - [0661] ii) the first and the second polypeptide comprise the mutations L251D, L314D and L432D, or
 - [0662] iii) the first and the second polypeptide comprise the mutations L251S, L314S and L432S, or
 - [0663] iv) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or

- [0664] v) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
- [0665] vi) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S.
- [0666] 52. A method for producing a dimeric polypeptide according to any one of items 1 to 51 comprising the following steps:
 - [0667] a) cultivating a mammalian cell comprising one or more nucleic acids encoding the dimeric polypeptide according to any one of items 1 to 51,
 - [0668] b) recovering the dimeric polypeptide from the cultivation medium, and
 - [0669] c) purifying the dimeric polypeptide with a protein A affinity chromatography.
- [0670] 53. Use of the mutation Y436A for increasing the binding of a dimeric polypeptide to protein A.
- [0671] 54. Use of the mutations H310A, H433A and Y436A for separating heterodimeric polypeptides from homodimeric polypeptides.
- [0672] 55. Use of the mutations L251D, L314D, L432D, or the mutations L251S, L314S, L432S for separating heterodimeric polypeptides from homodimeric polypeptides
- [0673] 56. Use of the mutations I253A, H310A and H435A in a first polypeptide in combination with the mutations H310A, H433A and Y436A in a second polypeptide for separating heterodimeric polypeptides comprising the first and the second polypeptide from homodimeric polypeptides.
- [0674] 57. Use of the mutations I253A, H310A and H435A in a first polypeptide in combination with the mutations L251D, L314D, L432D or the mutations L251S, L314S, L432S in a second polypeptide for separating heterodimeric polypeptides comprising the first and the second polypeptide from homodimeric polypeptides.
- [0675] 58. The use according to any one of items 53 to 57, characterized in that i) the first polypeptide further comprises the mutations Y349C, T366S, L368A and Y407V and the second polypeptide further comprises the mutations S354C and T366W, or ii) the first polypeptide comprises the mutations S354C, T366S, L368A and Y407V and the second polypeptide comprises the mutations Y349C and T366W.
- [0676] 59. A method of treatment of a patient suffering from ocular vascular diseases by administering a dimeric polypeptide according to any one of items 1 to 51 to a patient in the need of such treatment.
- [0677] 60. A dimeric polypeptide according to any one of items 1 to 51 for intravitreal application.
- [0678] 61. A dimeric polypeptide according to any one of items 1 to 51 for the treatment of vascular eye diseases.
- [0679] 62. A pharmaceutical formulation comprising a dimeric polypeptide according to any one of items 1 to 51 and optionally a pharmaceutically acceptable carrier.
- [0680] 63. Use of a dimeric polypeptide according to any one of items 1 to 51 for the transport of a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.

- [0681] 64. Use of a dimeric polypeptide according to any one of items 1 to 51 for the removal of one or more soluble receptor ligands from the eye.
- [0682] 65. Use of a dimeric polypeptide according to any one of items 1 to 51 for the treatment of eye diseases, especially of ocular vascular diseases.
- [0683] 66. Use of a dimeric polypeptide according to any one of items 1 to 51 for the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.
- [0684] 67. A dimeric polypeptide according to any one of items 1 to 51 for use in treating an eye disease.
- [0685] 68. A dimeric polypeptide according to any one of items 1 to 51 for use in the transport of a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.
- [0686] 69. A dimeric polypeptide according to any one of items 1 to 51 for use in the removal of one or more soluble receptor ligands from the eye.
- [0687] 70. A dimeric polypeptide according to any one of items 1 to 51 for use in treating eye diseases, especially ocular vascular diseases.
- [0688] 71. A dimeric polypeptide according to any one of items 1 to 51 for use in the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.
- [0689] 72. A method of treating an individual having an ocular vascular disease comprising administering to the individual an effective amount of a dimeric polypeptide according to any one of items 1 to 51.
- [0690] 73. A method for transporting a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation in an individual comprising administering to the individual an effective amount of a dimeric polypeptide according to any one of items 1 to 51 to transport a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.
- [0691] 74. A method the removal of one or more soluble receptor ligands from the eye in an individual comprising administering to the individual an effective amount of a dimeric polypeptide according to any one of items 1 to 51 to remove one or more soluble receptor ligands from the eye.
- [0692] 75. A method for the transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation in an individual comprising administering to the individual an effective amount of a dimeric polypeptide according to any one of items 1 to 51 to transport of one or more soluble receptor ligands from the intravitreal space to the blood circulation.
- [0693] 76. A method for transporting a soluble receptor ligand from the intravitreal space or the eye over the blood-ocular-barrier into the blood circulation in an individual comprising administering to the individual an effective amount of a dimeric polypeptide according to any one of items 1 to 51 to transport a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood circulation.

V. Examples

[0694] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

[0695] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

Methods

Electrospray Ionization Mass Spectrometry (ESI-MS)

[0696] Protein aliquots (50 µg) were deglycosylated by adding 0.5 µL N-Glycanase plus (Roche) and sodium phosphate buffer (0.1 M, pH 7.1) to obtain a final sample volume of 115 μ L. The mixture was incubated at 37° C. for 18 h. Afterwards for reduction and denaturing 60 µL 0.5 M TCEP (Pierce) in 4 M guanidine*HCl (Pierce) and 50 µL 8 M guanidine*HCl were added. The mixture was incubated at 37° C. for 30 min. Samples were desalted by size exclusion chromatography (Sepharose G-25, isocratic, 40% acetonitrile with 2% formic acid). ESI mass spectra (+ve) were recorded on a Q-TOF instrument (maXis, Bruker) equipped with a nano ESI source (TriVersa NanoMate, Advion). MS parameter settings were as follows: Transfer: Funnel RF, 400 Vpp; ISCID Energy, 0 eV; Multipole RF, 400 Vpp; Quadrupole: Ion Energy, 4.0 eV; Low Mass, 600 m/z; Source: Dry Gas, 8 L/min; Dry Gas Temperature, 160° C.; Collision Cell: Collision Energy, 10 eV; Collision RF: 2000 Vpp; Ion Cooler: Ion Cooler RF, 300 Vpp; Transfer Time: 120 µs; Pre Puls Storage, 10 μs; scan range m/z 600 to 2000. For data evaluation in-house developed software (MassAnalyzer) was used.

FcRn Surface Plasmon Resonance (SPR) Analysis

[0697] The binding properties of wild-type antibody and the mutants to FcRn were analyzed by surface plasmon resonance (SPR) technology using a BIAcore T100 instrument (BIAcore AB, Uppsala, Sweden). This system is well established for the study of molecular interactions. It allows a continuous real-time monitoring of ligand/analyte bindings and thus the determination of kinetic parameters in various assay settings. 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. If molecules bind to an immobilized ligand on the surface the mass increases, in case of dissociation the mass decreases. In the current assay, the FcRn receptor was immobilized onto a BIAcore CM5-biosensor chip (GE Healthcare Bioscience, Uppsala, Sweden) via amine coupling to a level of 400 Response units (RU). The assay was carried out at room temperature with PBS, 0.05% Tween20 pH 6.0 (GE Healthcare Bioscience) as running and dilution buffer. 200 nM of samples were injected at a flow rate of 50 μL/min at room temperature. Association time was 180 sec., dissociation phase took 360 sec. Regeneration of the chip surface was reached by a short injection of HBS-P, pH 8.0. Evaluation of SPR-data was performed by comparison of the biological response signal height at 180 sec. after injection and at 300 sec. after injection. The corresponding parameters are the RU max level (180 sec. after injection) and late stability (300 sec. after end of injection).

Protein a Surface Plasmon Resonance (SPR) Analysis

[0698] The assay is based on surface plasmon resonance spectroscopy. Protein A is immobilized onto the surface of

a SPR biosensor. By injecting the sample into the flow cells of the SPR spectrometer it forms a complex with the immobilized protein A resulting in an increasing mass on the sensor chip surface, and therefore to a higher response (as 1 RU is defined as 1 pg/mm²). Afterwards the sensor chip is regenerated by dissolving the sample-protein A-complex. The gained responses are then evaluated for the signal high in response units (RU) and the dissociation behavior [0699] Around 3500 response units (RU) of protein A (20 μg/mL) were coupled onto a CM5 chip (GE Healthcare) at pH 4.0 by using the amine coupling kit of GE Healthcare. [0700] The sample and system buffer was HBS-P+(0.01 M HEPES, 0.15 M NaCl, 0.005% Surfactant P20 Sterilefiltered, pH 7.4). Flow cell temperature was set to 25° C. and sample compartment temperature to 12° C. The system was primed with running buffer. Then, a 5 nM solutions of the sample constructs were injected for 120 seconds with a flow rate of 30 µL/min, followed by a 300 seconds dissociation phase. Then the sensor chip surface was regenerated by two 30 seconds long injections of Glycine-HCl pH 1.5 at a flow rate of 30 μ L/min. Each sample was measured as a triplicate. Bispecific Antibodies and their Respective Sequences

Description	Sequences
anti-VEGF/ANG2	SEQ ID NO: 34, SEQ ID
CrossMab IgG1 with	NO: 35, SEQ ID NO: 36,
IHH-AAA mutations	SEQ ID NO: 37
anti-VEGF/ANG2	SEQ ID NO: 52, SEQ ID
CrossMab IgG1 wild type	NO: 53, SEQ ID NO: 54,
(without IHH-AAA	SEQ ID NO: 55
mutations)	
anti-VEGF/ANG2	SEQ ID NO: 38, SEQ ID
CrossMab IgG1 with	NO: 39, SEQ ID NO: 40,
IHH-AAA mutations and	SEQ ID NO: 41
P329G LALA mutations	
anti-VEGF/ANG2	SEQ ID NO: 56, SEQ ID
CrossMab IgG1 with	NO: 57, SEQ ID NO: 58,
P329G LALA mutations	SEQ ID NO: 59
only (without IHH-AAA	
mutations)	
anti-VEGF/ANG2	SEQ ID NO: 42, SEQ ID
CrossMab IgG4 with	NO: 43, SEQ ID NO: 44,
IHH-AAA mutations and	SEQ ID NO: 45
with SPLE mutations	and the victorian
anti-VEGF/ANG2	SEQ ID NO: 46, SEQ ID
OAscFab IgG1 with IHH-	NO: 47, SEQ ID NO: 48
AAA mutations	GEO ID NO. 40 GEO ID
<vegf-ang-2></vegf-ang-2>	SEQ ID NO: 49, SEQ ID
OAscFab IgG4 with IHH-	NO: 50, SEQ ID NO: 51
AAA mutations and with SPLE mutations	
anti-VEGF/ANG2	SEO ID NO. 102 SEO ID
	SEQ ID NO: 102, SEQ ID
CrossMab IgG1 with HHY-AAA mutations	NO: 103, SEQ ID NO: 36, SEQ ID NO: 37
anti-VEGF/ANG2	SEQ ID NO: 104, SEQ ID
CrossMab IgG1 with	NO: 105, SEQ ID NO: 36,
HHY-AAA mutations and	SEQ ID NO: 37
P329G LALA mutations	SEQ ID NO. 37
anti-VEGF/ANG2	SEQ ID NO: 106, SEQ ID
CrossMab IgG4 with	NO: 107, SEQ ID NO: 58,
HHY-AAA mutations and	SEO ID NO: 59
with SPLE mutations	5EQ 1D 110. 33
<vegf-ang-2></vegf-ang-2>	SEQ ID NO: 108, SEQ ID
OAscFab IgG1 with	NO: 109, SEQ ID NO: 48
HHY-AAA mutations	1.5. 105, 522 25 1.0. 40
<vegf-ang-2></vegf-ang-2>	SEQ ID NO: 110, SEQ ID
OAscFab IgG4 with	NO: 111, SEQ ID NO: 51

-continued

Description	Sequences
HHY-AAA mutations and with SPLE mutations	

[0701] The term "with (the) mutation IHH-AAA" as used herein refers the combination of the mutations I253A (Ile253Ala), H310A (His310Ala), and H435A (His435Ala) in a constant heavy chain region of IgG1 or IgG4 subclass (numbering according to the Kabat EU index numbering system), the term "with (the) mutation HHY-AAA" as used herein refers the combination of the mutations H310A (His310Ala), H433A (His433Ala) and Y436A (Tyr436Ala) in a constant heavy chain region of IgG1 or IgG4 subclass (numbering according to the Kabat EU index numbering system), the term "with (the) mutation P329G LALA" as used herein refers to the combination of the mutations L234A (Leu234Ala), L235A (Leu235Ala) and P329G (Pro329Gly) in a constant heavy chain region of IgG1 subclass (numbering according to the Kabat EU index numbering system), and the term "with (the) mutation SPLE" as used herein refers to the combination of the mutations S228P (Ser228Pro) and L235E (Leu235Glu) in a constant heavy chain region of IgG4 subclass (numbering according to the Kabat EU index numbering system).

Description	Sequences
<igf-1r> IgG1 wt</igf-1r>	SEQ ID NO: 88
	SEQ ID NO: 89
<igf-1r> IgG1 with</igf-1r>	SEQ ID NO: 88
I253A, H310A, H435A	SEQ ID NO: 90
<igf-1r> IgG1 with</igf-1r>	SEQ ID NO: 88
M252Y, S254T, T256E	SEQ ID NO: 91
<igf-1r> IgG1 wt, KiH</igf-1r>	SEQ ID NO: 88
	SEQ ID NO: 92
	SEQ ID NO: 93
<igf-1r> IgG1 knob wt,</igf-1r>	SEQ ID NO: 88
hole I253A, H310A,	SEQ ID NO: 94
H435A	SEQ ID NO: 95
<igf-1r> IgG1 knob wt,</igf-1r>	SEQ ID NO: 88
hole H310A, H433A,	SEQ ID NO: 96
Y436A	SEQ ID NO: 97
<igf-1r> IgG1 knob wt,</igf-1r>	SEQ ID NO: 88
hole M252Y, S254T,	SEQ ID NO: 98
T256E	SEQ ID NO: 99
<igf-1r> IgG1 knob wt,</igf-1r>	SEQ ID NO: 88
hole L251D, L314D,	SEQ ID NO: 100
L432D	SEQ ID NO: 101
<igf-1r> IgG1 with</igf-1r>	SEQ ID NO: 88
H310A, H433A, Y436A	SEQ ID NO: 112

General

[0702] 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 acid residues 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

[0703] 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, N.Y. (1989). The molecular biological reagents were used according to the manufacturer's instructions.

Gene Synthesis

[0704] Desired gene segments were ordered according to given specifications at Geneart (Regensburg, Germany).

DNA Sequence Determination

[0705] 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

[0706] The GCG's (Genetics Computer Group, Madison, Wis.) 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

[0707] For the expression of the described antibodies expression vectors 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 used.

[0708] Beside the antibody expression cassette the vectors contained:

[0709] an origin of replication which allows replication of this vector in *E. coli*,

[0710] a β-lactamase gene which confers ampicillin resistance in *E. coli.*, and

[0711] the dihydrofolate reductase gene from *Mus musculus* as a selectable marker in eukaryotic cells.

[0712] The transcription unit of the antibody gene was composed of the following elements:

[0713] unique restriction site(s) at the 5' end,

[0714] the immediate early enhancer and promoter from the human cytomegalovirus,

[0715] in the case of the cDNA organization followed by the Intron A sequence,

[0716] a 5'-untranslated region of a human immunoglobulin gene,

[0717] a nucleic acid encoding an immunoglobulin heavy chain signal sequence,

[0718] a nucleic acid encoding the human antibody chain (wild-type or with domain exchange) either as cDNA or in genomic organization with the immunoglobulin exon-intron organization,

[0719] a 3' non-translated region with a polyadenylation signal sequence, and

[0720] unique restriction site(s) at the 3' end.

[0721] The nucleic acids encoding the antibody chains 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 vectors were prepared by vector preparation from transformed *E. coli* cultures (Nucleobond AX, Macherey-Nagel).

Cell Culture Techniques

[0722] 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.

[0723] The bispecific antibodies were expressed by transient co-transfection of the respective expression vectors in HEK29-F cells growing in suspension as described below.

Example 1

Expression and Purification

Transient Transfections in HEK293-F System

[0724] The monospecific and bispecific antibodies were generated by transient transfection with the respective vectors (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 FreeStyleTM 293 expression medium (Invitrogen) were transfected with a mix of the respective expression vectors and 293FectinTM or fectin (Invitrogen). For 2 L shake flask (Corning) HEK293-F cells were seeded at a density of 1*10⁶ cells/mL in 600 mL and incubated at 120 rpm, 8% CO₂. The day after the cells were transfected at a cell density of approx. 1.5*10⁶ cells/mL with approx. 42 mL mix of A) 20 mL Opti-MEM (Invitrogen) with 600 μg total vector 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 with 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

[0725] Bispecific antibodies were purified from cell culture supernatants by affinity chromatography using MabSelectSure-Sepharose™ (for non-IHH-AAA mutants) (GE Healthcare, Sweden) or KappaSelect-Agarose (for IHH-AAA mutants) (GE Healthcare, Sweden), hydrophobic interaction chromatography using butyl-Sepharose (GE Healthcare, Sweden) and Superdex 200 size exclusion (GE Healthcare, Sweden) chromatography.

[0726] Briefly, sterile filtered cell culture supernatants were captured on a MabSelectSuRe resin equilibrated (non-IHH-AAA mutations and wild-type antibodies) with PBS buffer (10 mM $\rm Na_2HPO_4$, 1 mM $\rm KH_2PO_4$, 137 mM $\rm NaCl$ and 2.7 mM $\rm KCl$, pH 7.4), washed with equilibration buffer and eluted with 25 mM sodium citrate at pH 3.0. The IHH-AAA mutants were captured on a KappaSelect resin equilibrated with 25 mM Tris, 50 mM $\rm NaCl$, pH 7.2, washed with equilibration buffer and eluted with 25 mM sodium

citrate pH 2.9. The eluted antibody fractions were pooled and neutralized with 2 M 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 (monospecific or 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 (monospecific or 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°

TABLE

Yields of bispecific <vegf-ang-2> antibodies</vegf-ang-2>								
	VEGF/ ANG2-0015 (without IHH-AAA mutation)	VEGF/ ANG2-0016 (with IHH-AAA mutation)	VEGF/ ANG2-0121 (with HHY-AAA mutation)					
`	64 μg/mL, (2 L = 128 mg) 118 mg (~70% monomer)	n.a. (2 L scale) n.a.	60.8 μg/mL (2 L = 121.60 mg) 100.5 mg (pool1 + pool2)					
Sure) Kappa Select	n.a.	117 mg (~83% monomer)	n.a.					
Butyl Sepharose	60 mg	57 mg	49 mg					
SEC	35 mg (>95% monomer)	38 mg (>95% monomer)	32.4 mg (>95% monomer)					

[0727] Purity and antibody integrity were analyzed after each purification step by CE-SDS using microfluidic Labchip technology (Caliper Life Science, USA). Five µL of protein solution was prepared for CE-SDS analysis using the HT Protein Express Reagent Kit according manufacturer's instructions and analyzed on Labchip GXII system using a HT Protein Express Chip. Data were analyzed using Labchip GX Software.

and 5.4 mM KCl, pH 7.4) running buffer at 25 $^{\circ}$ C. 25 μ g protein were injected on the column at a flow rate of 0.75 mL/min and eluted isocratic over 50 minutes.

[0729] Analogously the anti-VEGF/ANG2 antibodies VEGF/ANG2-0012 and VEGF/ANG2-0201 were prepared and purified with the following yields:

	VEGF/ANG2-0012 (with IHH-AAA mutation)	VEGF/ANG2-0201 (without IHH-AAA mutation)
titer //amount	_	36 μg/mL/72 mg
scale	2.1 L	2 L
protein A	_	66 mg
(MabSelectSure)		(~95% monomer)
KappaSelect	43 mg	_
	(~65% monomer)	
Butyl Sepharose		45 mg
SEC	14 mg	21 mg
		(>98% monomer)
yield hydroxylapatite	8.5 mg	
	(>98% monomer)	
total yield (recovery)	8.5 mg	21 mg
, , , , , , , , , , , , , , , , , , , ,	(20%)	(30%)
	(20%)	(30%)

[0730] Also the anti-VEGF/ANG2 bispecific antibodies anti-VEGF/ANG2 CrossMAb_IgG4 with IHH-AAA mutation and with SPLE mutation (SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45), anti-VEGF/ANG2 OAscFab IgG1 with IHH-AAA mutation (SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48), anti-VEGF/ANG2 OAscFab IgG4 with IHH-AAA mutation and with SPLE mutation (SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51), anti-VEGF/ANG2 CrossMab IgG1 with HHY-AAA mutation and P329G LALA mutation (SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 40, SEQ ID NO: 41), anti-VEGF/ ANG2 CrossMab IgG4 with HHY-AAA mutation and SPLE mutation (SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 44, SEQ ID NO: 45), anti-VEGF/ANG2 OAscFab IgG1 with HHY-AAA mutation (SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 48), and anti-VEGF/ANG2 OAscFab IgG4 with HHY-AAA mutation and SPLE mutation (SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 51) and also the anti-IGF-1R monospecific antibodies anti-IGF-1R wild-type (SEQ ID NO: 88, SEQ ID NO: 89), anti-IGF-1R IgG1 with IHH-AAA mutation (SEQ ID NO: 88, SEQ ID NO: 90), anti-IGF-1R IgG1 with YTE mutation (SEQ ID NO: 88, SEQ ID NO: 91), anti-IGF-1R IgG1 wild-type with KiH

TABLE

Removal of typical side products by different sequential purification steps determined by CE-SDS.												
	VEGF/ANG2-0015 % peak area* * analysis: C				VEGF/ANG2-0016 CE-SDS (Caliper Labchip GXII)							
purification step	mAb	³ / ₄ Ab	(HC)2	1/2 A b	(LC)2	LC	mAb	³ / ₄ A b	(HC)2	1/2 A b	(LC)2	LC
MAbSelect Sure Kappa Select Butyl-Sepharose	55.7 81.4	19 1.9	10.6 —	9.8 8.2	3.5	0.9	63 76.2	13.4	3.5 0.7	- 6.1 8.3	5.8 7.7	7.4 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.

[0728] The aggregate content of antibody samples was analyzed by high-performance SEC using a Superdex 200 analytical size-exclusion column (GE Healthcare, Sweden) in 2×PBS (20 mM Na₂HPO₄, 2 mM KH₂PO₄, 274 mM NaCl

mutation (SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 93), anti-IGF-1R IgG1 with KiH mutation and the IHH-AAA mutation in the hole chain (SEQ ID NO: 88, SEQ ID NO: 94, SEQ ID NO: 95), anti-IGF-1R IgG1 with KiH

mutation and the HHY-AAA mutation in the hole chain (SEQ ID NO: 88, SEQ ID NO: 96, SEQ ID NO: 97), anti-IGF-1R IgG1 with KiH mutation and the YTE mutation (SEQ ID NO: 88, SEQ ID NO: 98, SEQ ID NO: 99), anti-IGF-1R IgG1 with KiH mutation and the DDD mutation (SEQ ID NO: 88, SEQ ID NO: 100, SEQ ID NO: 101), and anti-IGF-1R IgG1 with HHY-AAA mutation (SEQ ID NO: 88, SEQ ID NO: 112) can be prepared and purified analogously.

Example 2

Analytics & Developability

Small-Scale DLS-Based Viscosity Measurement.

[0731] Viscosity measurement was essentially performed as described in (He, F. et al., Analytical Biochemistry 399 (2009) 141-143). 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 μ m filter plate and covered with paraffin oil. The apparent diameter of the latex beads is determined by dynamic light scattering at 25 η 0. The viscosity of the solution can be calculated as η = η 0(rh/rh,0) (η : viscosity; η 0: viscosity of water; rh: apparent hydrodynamic radius of the latex beads; rh,0: hydrodynamic radius of the latex beads in water).

[0732] To allow comparison of various samples at the same concentration, viscosity-concentration data were fitted with the Mooney equation (Equation 1) (Mooney, M., Colloid. Sci., 6 (1951) 162-170; Monkos, K., Biochem. Biophys. Acta 304 (1997) 1339) 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)

[0733] Results are shown in FIG. 2: VEGF/ANG2-0016 with IHH-AAA mutation in the Fc-region shows a lower viscosity at all measured temperatures compared to VEGF/ANG2-0015 without the IHH-AAA mutation in the Fc-region.

DLS Aggregation Onset Temperature

[0734] Samples are prepared at a concentration of 1 mg/mL in 20 mM histidine/histidine hydrochloride, 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 paraffin 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 FIG. 3. In FIG. 3 the aggregation of VEGF/ANG2-0015 without the IHH-AAA mutation versus VEGF/ANG2-0016 with IHH-AAA mutation in the Fc-region is shown. VEGF/ANG2-0016 showed an aggregation onset

temperature of 61° C. whereas VEGF/ANG2-0015 without the IHH-AAA mutation showed an onset temperature of 60° C.

DLS Time-Course

[0735] Samples are prepared at a concentration of 1 mg/mL in 20 mM histidine/histidine hydrochloride, 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 paraffin 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 VEGF/ANG2-0015 and VEGF/ANG2-0016. Seven day storage at 40° C. at 100 mg/mL

[0736] 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 the Table below and FIG. 4, which show that VEGF/ANG2-0015 (without IHH-AAA mutation) shows a higher reduction of the main peak and a higher HMW increase compared to VEGF/ANG2-0016 (with IHH-AAA mutation). Surprisingly VEGF/ANG2-0016 (with IHH-AAA mutation) showed a lower aggregation tendency compared to VEGF/ANG2-0015 (without IHH-AAA mutation).

TABLE

Delta Main-, HMW and LMW peaks after 7 d at 40° C.				
	delta_area %(40° C(-80° C.))			
	main Peak	HMW	LMW	
VEGF/ANG2-0015	-3.56	2.89	0.67	
(without IHH-AAA mutation) VEGF/ANG2-0016 (with IHH-AAA mutation)	-1.74	1.49	0.25	

[0737] The functional analysis of anti-VEGF/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, FegammaR and FeRn

VEGF Isoforms Kinetic Affinity Including Assessment of Species-Cross-Reactivity

[0738] Around 12,000 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 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 seconds 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 seconds at a flow of 30 µL/min starting with 300 nM in 1:3 dilutions. The dissociation phase was monitored for up to 1200 seconds and triggered by switching from the sample solution to running buffer. The surface was regenerated by 60 seconds 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 below.

ANG2 Solution Affinity Including Assessment of Species-Cross-Reactivity

[0739] 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 anti-VEGF/ANG2 antibody, kept at a constant concentration, with a ligand (=ANG2) at varying concentrations. Maximum possible resonance units (e.g. 17,000 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 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 flow-cell containing the immobilized anti-VEGF/ANG2 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 anti-VEGF/ANG2 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 seconds washing with a 0.85% $\rm H_3PO_4$ solution at a flow rate of 30 $\rm \mu L/min$. Bulk refractive index differences were corrected by subtracting the response obtained from a blank-coupled surface. Results are shown in below.

FcRn Steady State Affinity

[0740] 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, pH 5.0) 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, 250 nM, and 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 seconds association time. The surface was regenerated by injected PBS-T pH 8 for 60 seconds 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 were plotted against the analyzed concentrations, yielding a dose-response curve. Based on a 2-parametric fit, the upper asymptote is calculated, allowing the determination of the halfmaximal RU value and hence the affinity. Results are shown in FIG. 5 and the Table below. Analogously the affinity to Cynomolgus, mouse and rabbit FcRn can be determined.

FcgammaRIIIa Measurement

[0741] For FegammaRIIIa measurement a direct binding assay was used. Around 3,000 resonance units (RU) of the capturing system (1 µg/mL Penta-His; Qiagen) were coupled on a CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by 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 seconds at a flow of 5 µL/min. Binding was measured by injection of 100 nM of bispecific antibody or monospecific control antibodies (anti-digoxygenin antibody for IgG1 subclass and an IgG4 subclass antibody) for 180 seconds at a flow of 30 μL/min. The surface was regenerated by 120 seconds 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 FegammaRIa and FegammaRIIa binding can be determined. Results are shown in FIG. 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 Anti-VEGF/ANG2 Antibodies

[0742] Around 3,500 resonance units (RU) of the capturing system (10 μ g/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 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.

[0743] The bispecific antibody was captured by injecting a 10 nM solution for 60 seconds at a flow of 5 μ L/min. Independent binding of each ligand to the bispecific antibody was analyzed by determining the active binding capacity for each ligand, either added sequentially or simultaneously (flow of 30 μ L/min):

[0744] 1. Injection of human VEGF with a concentration of 200 nM for 180 seconds (identifies the single binding of the antigen).

[0745] 2. Injection of human ANG2 with a concentration of 100 nM for 180 seconds (identifies single binding of the antigen).

[0746] 3. Injection of human VEGF with a concentration of 200 nM for 180 seconds followed by an additional injection of human ANG2 with a concentration of 100 nM for 180 seconds (identifies binding of ANG2 in the presence of VEGF).

[0747] 4. Injection of human ANG2 with a concentration of 100 nM for 180 seconds followed by an additional injection of human VEGF with a concentration of 200 nM (identifies binding of VEGF in the presence of ANG2).

[0748] 5. Co-injection of human VEGF with a concentration of 200 nM and of human ANG2 with a concentration of 100 nM for 180 seconds (identifies the binding of VEGF and of ANG2 at the same time).

[0749] The surface was regenerated by 60 seconds washing with a 3 M MgCl $_2$ solution at a flow rate of 30 $\mu L/min.$ Bulk refractive index differences were corrected by subtracting the response obtained from a goat anti-human IgG surface.

[0750] 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 the Table below, where both antibodies VEGF/ANG2-0016, VEGF/ANG2-0012 are shown to be able to bind mutual independently to VEGF and ANG2.

Assessment of Simultaneous VEGF- and ANG2-Binding to the Anti-VEGF/ANG2 Antibodies

[0751] First, around 1,600 resonance units (RU) of VEGF (20 $\mu g/mL$) were coupled on a CM4 chip (GE Healthcare BR-1005-34) at pH 5.0 by using an amine coupling kit supplied by GE Healthcare. The sample and system buffer was PBS-T (10 mM phosphate buffered saline including 0.05% Tween20) pH 7.4. Flow cell was set to 25° C. and sample block to 12° C. and primed with running buffer twice. Second, 50 nM solution of the bispecific antibody was injected for 180 seconds at a flow of 30 $\mu L/min$. Third, hANG2 was injected for 180 seconds at a flow of 30 $\mu L/min$. The binding response of hANG2 depends from the amount of the bispecific antibody bound to VEGF and shows simultaneous binding. The surface was regenerated by 60 seconds washing with a 0.85% $\rm H_3PO_4$ solution at a flow rate of 30 $\mu L/min$. Simultaneous binding is shown by an additional

specific binding signal of hANG2 to the previous VEGF bound anti-VEGF/ANG2 antibodies. For both bispecific antibodies VEGF/ANG2-0015 and VEGF/ANG2-0016 simultaneous VEGF- and ANG2-binding to the anti-VEGF/ANG2 antibodies could be detected (data not shown).

TABLE

Results:	Kinetic affiniti	es to VEGF isof	orms from diffe	rent species
	VEGF/	VEGF/	VEGF/	VEGF/
	ANG2-	ANG2-	ANG2-	ANG2-
	0015 -	0016 -	0012 -	0201 -
	apparent	apparent	apparent	apparent
	affinity	affinity	affinity	affinity
human	≤1 pM (out	≤1 pM (out	≤1 pM (out	≤1 pM (out
VEGF 121	of BIAcore	of BIAcore	of BIAcore	of BIAcore
	specifi-	specifi-	specifi-	specifi-
	cation)	cation)	cation)	cation)
mouse	no binding	no binding	no binding	no binding
VEGF 120				
			24.37	25 26
Rat VEGF 164	13 nM	14 nM	24 nM	35 nM

TABLE

Results: Solution affinities to ANG2				
	VEGF/	VEGF/	VEGF/	VEGF/
	ANG2-0015	ANG2-0016	ANG2-0012	ANG2-0201
	KD	KD	KD	KD
	[nM]	[nM]	[nM]	[nM]
human ANG2	8	20	20	n.d.
cyno ANG2	5	13	10	n.d.
mouse ANG2	8	13	8	n.d.
rabbit ANG2	4	11	8	n.d.

TABLE

Results: Affinity to FcRn of anti-VEGF/ANG2 antibodies				
	VEGF/ ANG2-0015 [affinity]	VEGF/ ANG2-0016 [affinity]	VEGF/ ANG2-0012 [affinity]	VEGF/ ANG2-0201 [affinity]
human FcRn cynomolgus FcRn mouse FcRn	0.8 µМ 0.9 µМ 0.2 µМ	no binding no binding no binding	no binding no binding no binding	0.8 μM 1.0 μM 0.2 μM

TABLE

Results Binding to FcgammaRI-IIIa				
	VEGF/	VEGF/	VEGF/	VEGF/
	ANG2-0015	ANG2-0016	ANG2-0012	ANG2-0201
FcγRIa	no binding	no binding	binding	binding
FcγRIIa	no binding	no binding	no binding	binding
FcγRIIIa	no binding	no binding	no binding	binding

TABLE

Results: Independent binding of VEGF- and ANG2 to anti-VEGF/ANG2 antibodies					
	ANG2 [RUmax]	VEGF [RUmax]	first VEGF then ANG2 [RUmax]	first ANG2 then VEGF [RUmax]	Co- injection ANG2 + VEGF [RUmax]
VEGF/ ANG2-0016 VEGF/	174 143	50	211 178	211 177	211 178
ANG2-0012	143	73	1/6	1//	1/6

Example 4

Mass Spectrometry

[0752] This section describes the characterization of anti-VEGF/ANG2 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) anti-VEGF/ANG2 antibodies. The IdeS-digestion was performed with 100 µg purified antibody incubated with 2 µg IdeS protease (Fabricator) in 100 mmol/L NaH₂PO₄/Na₂HPO₄, pH 7.1 at 37° C. for 5 h. Subsequently, the antibodies were deglycosylated with N-Glycosidase F, Neuraminidase and 0-glycosidase (Roche) in 100 mmol/L NaH₂PO₄/Na₂HPO₄, pH 7.1 at 37° C. for up to 16 hours 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).

[0753] The masses obtained for the IdeS-digested, degly-cosylated (Table below), or intact, degly-cosylated (Table below) molecules correspond to the predicted masses deduced from the amino acid sequences for the anti-VEGF/ANG2 antibodies consisting of two different light chains LC_{ANG2} and $LC_{Lucentis}$, and two different heavy chains HC_{ANG2} and $HC_{Lucentis}$.

TABLE

Masses of the deglycosylated and IdeS-digested bispecific anti-VEGF/ANG2 antibodies VEGF/ANG2-0201 (without IHH-AAA mutation) and VEGF/ANG2-0012 (with IHH-AAA mutation)

	F(ab') ₂ of the anti- VEGF/ANG2 antibody		deglycosylated Fc- region of the anti- VEGF/ANG2 antibody	
sample	predicted average mass [Da]	observed average mass [Da]	predicted average mass [Da]	observed average mass [Da]
VEGF/ ANG2-0201	99360.8	99360.7	47439.2	47430.1
VEGF/ ANG2-0012	99360.8	99361.1	47087.7	47082.0

TABLE

Masses of the deglycosylated anti-VEGF/ANG2 antibodies VEGF/ANG2-0016 (with IHH-AAA mutation) and VEGF/ANG2-0015 (without IHH-AAA mutation)

	deglycosylated anti-VEGF/ANG2 antibody		
	predicted average mass [Da]	observed average mass [Da]	
VEGF/ ANG2-0016	146156.9	146161.2	
VEGF/ ANG2-0015	146505.3	146509.4	

Example 5

FeRn Chromatography

Coupling to Streptavidin Sepharose:

[0754] 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:

[0755] Conditions:

[0756] column dimensions: 50 mm×5 mm

[0757] bed height: 5 cm

[0758] loading: 50 μg sample

[0759] equilibration buffer: 20 mM MES, with 150 mM NaCl, adjusted to pH 5.5

[0760] elution buffer: 20 mM Tris/HCl, with 150 mM NaCl, adjusted to pH 8.8

[0761] elution: 7.5 CV equilibration buffer, in 30 CV to 100% elution buffer, 10 CV elution buffer

Human FcRn Affinity Column Chromatography

[0762] In the following Table retention times of anti-VEGF/ANG2 antibodies on affinity columns comprising human FcRn are given. Data were obtained using the conditions above.

TABLE

Results: retention times of anti-VEGF/ANG2 antibodies			
antibody	retention time [min]		
VEGF/ANG2-0015 (without IHH-AAA mutation) VEGF/ANG2-0201 (without IHH-AAA mutation) VEGF/ANG2-0012 (with IHH-AAA mutation) VEGF/ANG2-0016 (with IHH-AAA mutation)	78.5 78.9 2.7 (void-peak) 2.7 (void-peak)		

Example 6

Pharmacokinetic (PK) Properties of Antibodies with IHH-AAA Mutation

[0763] PK Data with FcRn Mice Transgenic for Human FcRn

In life phase:

[0764] The study included female C57BL/6J mice (background); mouse FcRn deficient, but hemizygous transgenic for human FcRn (huFcRn, line 276-/tg)

Part 1:

[0765] All mice were injected once intravitreally into the right eye with 2 μ L/animal of the appropriate solution (i.e. 21 μ g compound/animal (VEGF/ANG2-0015 (without IHH-AAA mutation)) or 23.6 μ g compound/animal (VEGF/ANG2-0016 (with IHH-AAA mutation)).

[0766] 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. [0767] 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.

[0768] Blood was collected via the retrobulbar venous plexus of the contralateral eye from each animal for the determination of the compound levels in serum.

[0769] Serum samples of at least 50 μ L were obtained from blood after 1 hour at RT by centrifugation (9,300×g) 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:

[0770] All mice were injected once intravenously via the tail vein with 200 μ L/animal of the appropriate solution (i.e. 21 μ g compound/animal (VEGF/ANG2-0015 (without IHH-AAA mutation)) or 23.6 μ g compound/animal (VEGF/ANG2-0016 (with IHH-AAA mutation)).

[0771] 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

[0772] Serum samples of at least 50 μ L were obtained from blood after 1 hour at RT by centrifugation (9,300×g) 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)

[0773] 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 thaw-

ing, the eyes were washed with 1 mL cell washing buffer once (Bio-Rad, Bio-Plex Cell Lysis Kit, Cat. No. 171-304011). In the following step, 500 μL of freshly prepared cell lysis buffer were added and the eyes were grinded using a 1.5 mL tissue grinding pestle (Kimble Chase, 1.5 mL 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 4,500 g. After centrifuging the supernatant was collected and stored at -20° C. until further analysis in the quantification ELISA.

Analysis

[0774] The concentrations of the anti-VEGF/ANG2 antibodies in mice serum and eye lysates were determined with an enzyme linked immunosorbent assay (ELISA)

[0775] For quantification of anti-VEGF/ANG2 antibodies in mouse serum samples and eye lysates, a standard solidphase serial sandwich immunoassay with biotinylated and digoxigenylated monoclonal antibodies used as capture and detection antibodies was performed. To verify the integrity of the bispecificity of the analyte the biotinylated capture antibody recognizes the VEGF-binding site whereas the digoxigenylated detection antibody will bind to the 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.

[0776] In a first step the SA-MTP was coated with 100 μ L/well of biotinylated capture antibody solution (mAb<Id<VEGF>>M-2.45.51-IgG-Bi(DDS), anti-idiotypic antibody) with a concentration of 1 μ g/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.

[0777] After coating the SA-MTP with capture antibody, the plate was washed three times with washing buffer and 300 $\mu L/well$. Subsequently 100 $\mu L/well$ 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 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 100 μL/well of the first detection antibody (mAb<Id-<ANG2>>M-2.6.81-IgG-Dig(XOSu), anti-idiotypic antibody) with a concentration of 250 ng/mL was added to the SA-MTP. Again, the plate was incubated for one hour at 500 rpm on a shaker. After washing, 100 µL/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

[0778] The pharmacokinetic parameters were calculated by non-compartmental analysis, using the pharmacokinetic evaluation program WinNonlinTM (Pharsight), version 5.2.1.

Results:

A) Serum Concentrations

[0779] Results for serum concentrations are shown in the following Tables and FIGS. 7B to 7C.

TABLE

VEGF/ANG2-0015 (without IHH-AAA mutation): Comparison of serum concentrations after intravitreal and intravenous application				
ID	serum concentration after intravitreal application average conc. [µg/mL]	serum concentration after intravenous application average conc. [µg/mL]		
1 h		17.7		
1 11		17.7		
2 h	9.8	17.7		
	9.8 10.4	12.1		
2 h				
2 h 7 h	10.4	12.1		
2 h 7 h 24 h	10.4 6.4	12.1 8.3		

TABLE

VEGF/ANG2-0016 (with IHH-AAA mutation): Comparison of serum

concentrations after intravitreal and intravenous application			
ID	serum concentration after intravitreal application average conc. [µg/mL]	serum concentration after intravenous application average conc. [µg/mL]	
1 h		18.4	
2 h	7.0		
7 h	8.7	10.0	
24 h	2.2	3.3	
48 h	1.0	1.0	
96 h	0.1	0.1	
168 h	0.0	0.0	

TABLE

VEGF/ANG2-0015 (without IHH-AAA mutation) and VEGF/ANG2-0016 (with IHH-AAA mutation): Comparison of serum concentrations after intravitreal application)

ID	VEGF/ANG2-0015 (without IHH-AAA mutation) average conc. [µg/mL]	VEGF/ANG2-0016 (with IHH-AAA mutation) average conc. [µg/mL]
2 h	9.8	7.0
7 h	10.4	8.7
24 h	6.4	2.2
48 h	6.5	1.0
96 h	3.4	0.1
168 h	2.9	0.0

TABLE

VEGF/ANG2-0015 (without IHH-AAA mutation) and VEGF/ANG2-0016 (with IHH-AAA mutation): Comparison of serum concentrations after intravenous application

ID	VEGF/ANG2-0015 (without IHH-AAA mutation) average conc. [µg/mL]	VEGF/ANG2-0016 (with IHH-AAA mutation) average conc. [µg/mL]
1 h	17.7	18.4
7 h	12.1	10.0
24 h	8.3	3.3
48 h	6.9	1.0
96 h	4.1	0.1
168 h	2.7	0.0

Results:

B) Concentrations in Eye-Lysates of Left and Right Eyes

[0780] Results for concentrations in eye lysates are shown in the following Tables and FIGS. 7D to 7E.

TABLE

Concentrations of VEGF/ANG2-0015 (without IHH-AAA mutation) in eye lysates after intra vitreal application into right eye mean conc. values from n=6 mice

	ID	mean conc. [ng/mL]	
96 h	left eye right eye	8.7 46.1	_
168 h	left eye right eye t	4.3 12.9	

TABLE

Concentrations of VEGF/ANG2-0015 (without IHH-AAA mutation) in eye lysates after intravenous application mean conc. values from n = 5 mice

	ID	mean conc. [ng/mL]	
96 h	left eye right eye	4.2 7.5	
168 h	left eye right eye	3.4 6.1	

TABLE

Concentrations of VEGF/ANG2-0016 (with IHH-AAA mutation) in eye lysates after intra vitreal application into right eye mean conc. values from n=5 mice

	ID	mean conc. [ng/mL]
96 h	left eye right eye	0.3 34.5
168 h	left eye right eye	0.1 9.0

TABLE

Concentrations of VEGF/ANG2-0016 (with IHH-AAA mutation) in eye lysates after intravenous application mean conc. values from n = 5 mice

	ID	mean conc. [ng/mL]
96 h	left eye right eye	0.0 0.1
168 h	left eye right eye	0.0 0.1

Summary of Results:

[0781] After intravitreal application the bispecific anti-VEGF/ANG2antibody as reported herein VEGF/ANG2-0016 (with IHH-AAA mutation) shows similar concentrations (after 96 and 168 hours) in the eye lysates as compared to the bispecific anti-VEGF/ANG2 antibody without IHH-AAA mutation VEGF/ANG2-0015.

[0782] Also after intravitreal application the bispecific anti-VEGF/ANG2 antibody as reported herein VEGF/ANG2-0016 (with IHH-AAA mutation) shows in addition a faster clearance and shorter half-life in the serum as compared to the bispecific anti-VEGF/ANG2 antibody without IHH-AAA mutation VEGF/ANG2-0015.

Example 7

Mouse Cornea Micropocket Angiogenesis Assay

[0783] To test the anti-angiogenic effect bispecific anti-VEGF/ANG2antibody with the respective VEGF binding VH and VL of SEQ ID NO: 20 and 21 and the ANG2 binding VH and VL of SEQ ID NO: 28 and 29 on VEGFinduced angiogenesis in vivo, a mouse corneal angiogenesis assay was performed. 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.

[0784] 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 VEGF/ANG2-0015 (without IHH-AAA mutation) which only differs from VEGF/ANG2-0016 by the IHH-AAA

mutation and has the same VEGF and ANG2 binding 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.

Example 8

Pharmacokinetic (PK) Properties of Antibodies with HHY-AAA Mutation

[0785] PK Data with FcRn Mice Transgenic for Human FcRn

In life phase:

[0786] The study included female C57BL/6J mice (background); mouse FcRn deficient, but hemizygous transgenic for human FcRn (huFcRn, line 276-/tg)

Part 1:

[0787] All mice were injected once intravitreally into the right eye with the appropriate solution of IGF-1R 0033, IGF-1R 0035, IGF-1R 0045 (i.e. 22.2 µg compound/animal of IGF-1R 0033, 24.4 µg compound/animal IGF-1R 0035, 32.0 µg compound/animal IGF-1R and 32.0 µg compound/animal of IGF-1R 0045).

[0788] Thirteen mice were allocated to 2 groups with 6 and 7, respectively, 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.

[0789] 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.

[0790] Blood was collected via the retrobulbar venous plexus of the contralateral eye from each animal for the determination of the compound levels in serum.

[0791] Serum samples of at least 50 μ L were obtained from blood after 1 hour at RT by centrifugation (9,300×g) 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:

[0792] All mice were injected once intravenously via the tail vein with the appropriate solution of IGF-1R 0033, IGF-1R 0035, IGF-1R 0045 (i.e. 22.2 µg compound/animal of IGF-1R 0033, 24.4 µg compound/animal IGF-1R 0035, 32.0 µg compound/animal IGF-1R and 32.0 µg compound/animal of IGF-1R 0045).

[0793] Twelve mice were allocated to 2 groups with 6 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.

[0794] Serum samples of at least 50 μ L were obtained from blood after 1 hour at RT by centrifugation (9,300×g) at 4° C. for 3 min. Serum samples were frozen directly after centrifugation and stored frozen at -80° C. until analysis.

Preparation of Cell Lysis Buffer

[0795] Carefully mix 100 μ L factor 1, 50 μ L factor 2 and 24.73 mL Cell Lysis buffer (all from Bio-Rad, Bio-Plex Cell Lysis Kit, Cat. No. 171-304011) and add 125 μ L PMSF-solution (174.4 mg phenylmethylsulfonylfluoride diluted in 2.0 mL DMSO).

Preparation of Whole Eye Lysates (Mice)

[0796] 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 thawing, the eyes were washed with 1 mL cell washing buffer once (Bio-Rad, Bio-Plex Cell Lysis Kit, Cat. No. 171-304011). In the following step 500 μL of freshly prepared cell lysis buffer were added and the eyes were grinded using a 1.5 mL tissue grinding pestle (VWR Int., Art. No. 431-0098). 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 (Serum)

[0797] For quantification of antibodies in mouse serum sample, a standard solid-phase serial sandwich immunoassay with biotinylated and digoxigenated monoclonal antibodies used as capture and detection antibodies is performed. Serum accounts for about 50% of the full blood sample volume.

[0798] More detailed, concentrations of the antibodies in mouse serum samples were determined by a human-IgG (Fab) specific enzyme linked immunosorbent assay. Streptavidin coated microtiter plates were incubated with the biotinylated anti-human Fab(kappa) monoclonal antibody M-1. 7.10-IgG as capture antibody diluted in assay buffer for one hour at room temperature with agitation. After washing three times with phosphate-buffered saline-polysorbate 20 (Tween20), serum samples at various dilutions were added followed by second incubation for one hour at room temperature. After three repeated washings bound antibody was detected by subsequent incubation with the anti-human Fab(CH1) monoclonal antibody M-1.19.31-IgG conjugated to digoxigenin, followed by an anti-digoxigenin antibody conjugated to horseradish peroxidase (HRP). ABTS (2.2'azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); Roche Diagnostics GmbH, Mannheim, Germany) was used as HRP substrate to form a colored reaction product. Absorbance of the resulting reaction product was read at 405 nm (ABTS; reference wavelength: 490 nm).

[0799] All samples, positive and negative control samples were analyzed in replicates and calibrated against an antibody standard provided.

Analysis (Eye Lysate)

[0800] The concentrations of the analytes in mouse eye lysate samples were determined using a qualified electrochemiluminescence immunoassay (ECLIA) method based

on the ELECSYS® instrument platform (Roche Diagnostics GmbH, Mannheim, Germany) under non-GLP conditions.

[0801] The undiluted supernatant (eye lysates) was incubated with capture and detection molecules for 9 min. at 37° C. Biotinylated anti-human-Fab(kappa) monoclonal antibody M-1.7.10-IgG was used as capture molecule and a ruthenium(Ietris(bispyridyl)₃²⁺ labeled anti-human-Fab (CH1) monoclonal antibody M-1.19.31-IgG was used for detection. Streptavidin-coated magnetic microparticles were added and incubated for additional 9 min. at 37° C. to allow binding of preformed immune complexes due to biotinstreptavidin interactions. The microparticles were magnetically captured on an electrode and a chemiluminescent signal generated using the co-reactant tripropyl amine (TPA). The gained signal was measured by a photomultiplier detector.

TABLE

Standard chart IGF-1R 0033					
	concen- tration [ng/mL]	signal mean counts	standard devia- tion signal counts	serum- conc. [ng/mL]	Recovery
standard sample 9	0	1038	46	_	_
standard sample 8	0.686	2682	105	0.675	98
standard sample 7	2.06	6275	791	2.06	100
standard sample 6	6.17	15907	316	6.23	101
standard sample 5	18.5	45455	1238	18.8	102
standard sample 4	55.6	133940	949	55.7	100
standard sample 3	167	388069	2929	165	99
standard sample 2	500	1129804	16777	503	101
standard sample 1	1500	2956965	60287	1499	100

TABLE

Standard chart IGF-1R 0035					
	concen- tration [ng/mL]	signal mean counts	standard devia- tion signal counts	serum- conc. [ng/mL]	Recovery
standard sample 9	0	1024	63		—
standard sample 8	0.686	2817	38	0.681	99
standard sample 7	2.06	6451	39	2.08	101
standard sample 6	6.17	17100	319	6.13	99
standard sample 5	18.5	49693	713	18.6	100
standard sample 4	55.6	146746	2575	56.1	101
standard sample 3	167	423597	5068	165	99
standard sample 2	500	1224244	11655	502	100
standard sample 1	1500	3144901	44536	1499	100

TABLE

Standard chart IGF-1R 0045					
	concen- tration [ng/mL]	signal mean counts	standard devia- tion signal counts	serum- conc. [ng/mL]	Recov- ery [%]
standard sample 9 standard sample 8 standard sample 7	0 0.686 2.06	1339 3108 7032	545 61 189	 0.622 1.93	— 91 94

TABLE-continued

Standard chart IGF-1R 0045					
			standard		
			devia-		
	concen-	signal	tion	serum-	Recov-
	tration	mean	signal	conc.	ery
	[ng/mL]	counts	counts	[ng/mL]	[%]
standard sample 6	6.17	19175	750	6.10	99
•					
standard sample 5	18.5	55526	823	18.7	101
standard sample 4	55.6	158591	5412	55.7	100
standard sample 3	167	456316	28759	167	100
standard sample 2	500	1274801	47532	499	100
standard sample 1	1500	3280452	239523	1501	100

Results:

A) Serum Concentrations

[0802] Results for serum concentrations are shown in the following Tables and FIG. 17.

TABLE

IGF-1R 0033 (without HHY-AAA mutation): Comparison of serun	1
concentrations after intravitreal and intravenous application	

ID	serum concentration after intravitreal application average conc. [µg/mL]	serum concentration after intravenous application average conc. [µg/mL]
1 h	n.d.	34.7
2 h	5.9	n.d.
7 h	11.1	24.7
24 h	4.4	13.6
48 h	7.8	12.6
96 h	2.1	8.9
168 h	2.9	6.2

(n.d. = not determined)

TABLE

IGF-1R 0035 (with HHY-AAA mutation in one Fe-region polypeptide): Comparison of serum concentrations after intravitreal and intravenous application

ID	serum concentration after intravitreal application average conc. [µg/mL]	serum concentration after intravenous application average conc. [µg/mL]
1 h	n.d.	24.5
2 h	7.3	n.d.
7 h	7.9	16.1
24 h	2.3	5.7
48 h	1.7	2.9
96 h	0.3	0.6
168 h	0.1	0.2

TABLE

IGF-1R 0045 (with HHY-AAA mutation in both Fc-region polypeptides): Comparison of serum concentrations after intravitreal and intravenous application

ID	serum concentration after intravitreal application average conc. [µg/mL]	serum concentration after intravenous application average conc. [μg/mL]
1 h	n.d.	40.5
2 h	13.2	n.d.
7 h	9.6	21.7
24 h	2.2	5.1
48 h	0.9	0.7
96 h	0.05	0.03
168 h	0.01	BLQ

(BLQ = below limit of quantitation)

TABLE

Comparison of serum concentrations after intravenous application of antibodies IGF-1R 0033, 0035 and 0045 normalized to 1 µg applied antibody

ID	IGF-1R 0033 average conc	IGF-1R 0035 . [ng/mL/μg applied	
1 h	1564	1006	1266
7 h	1114	659	679
24 h	613	234	160
48 h	569	118	21
96 h	399	26	1
168 h	280	7	0

Results:

B) Concentrations in Eye-Lysates of Left and Right Eyes

[0803] Results for concentrations in eye lysates are shown in the following Tables and FIGS. 18 to 20.

TABLE

Concentrations of IGF-1R 0033 (without HHY-AAA mutation) in eye lysates after intravitreal application into the right eye mean conc. values from $n=7\ (96\ h)$ and $n=6\ (196\ h)$ mice

	ID	mean conc. [ng/mL]
96 h	left eye right eye	3.3 99.5
168 h	left eye right eye	5.2 144.9

TABLE

Concentrations of IGF-1R 0033 (without HHY-AAA mutation) in eye lysates after intravenous application mean conc. values from $n=5\ (96\ h)$ and $n=6\ (196\ h)$ mice

	ID	mean conc. [ng/mL]
96 h	left eye right eye	12.7 8.5

TABLE-continued

Concentrations of IGF-1R 0033 (without HHY-AAA mutation) in eye lysates after intravenous application mean conc. values from $n=5\ (96\ h)$ and $n=6\ (196\ h)$ mice

	ID	mean conc. [ng/mL]
168 h	left eye right eye	9.7 BLQ

(BLQ = below limit of quantitation)

TABLE

Concentrations of IGF-1R 0035 (with the HHY-AAA mutation in one Fc-region polypeptide) in eye lysates after intravitreal application into the right eye mean conc. values from n=6 mice

	ID	mean conc. [ng/mL]
96 h	left eye right eye	1.1 169.2
168 h	left eye right eye	0.3 114.7

TABLE

Concentrations of IGF-1R 0035 (with the HHY-AAA mutation in one Fc-region polypeptide) in eye lysates after intravenous application mean conc. values from n=6 mice

	ID	mean conc. [ng/mL]
96 h	left eye right eye	3.7 1.7
168 h	left eye right eye	1.4 0.3

(BLQ = below limit of quantitation)

TABLE

Concentrations of IGF-1R 0045 (with the HHY-AAA mutation in both Fc-region polypeptides) in eye lysates after intravitreal application into the right eye mean conc. values from n = 6 mice

	ID	mean conc. [ng/mL]
96 h	left eye right eye	1.4 322.6
168 h	left eye right eye	1.4 156.8

TABLE

Concentrations of IGF-1R 0045 (with the HHY-AAA mutation in both Fc-region polypeptides) in eye lysates after intravenous application mean conc. values from n = 6 (96 h) and n = 5 (196 h) mice

	ID	mean conc. [ng/mL]
96 h	left eye right eye	3.6 1.3
168 h	left eye right eye	0.8 0.4

(BLQ = below limit of quantitation)

TABLE

Concentrations of IGF-1R 0033, 0035 and 0045 in
eye lysates after intravitreal application into
the right eye normalized to 1 µg applied antibody

IGF-1R 0033 IGF-1R 0035 IGF-1R 004
ID mean conc. [ng/mL]

96 h left eye 0.15 0.05 0.04

	ID		IGF-1R 0035 lean conc. [ng/m]	
96 h	left eye	0.15	0.05	0.04
	right eye	4.48	6.93	10.08
168 h	left eye	0.24	0.01	0.04
	right eye	6.53	4.70	4.90

Summary of Results:

[0804] After intravitreal application the anti-IGF-1R anti-bodies 0035 and 0045 as reported herein (with one sided or both sided HHY-AAA mutation) shows similar concentrations (after 96 and 168 hours) in the eye lysates as compared to the anti-IGF-1R antibody without HHY-AAA mutation (IGF-1R 0033).

[0805] Also after intravitreal application the anti-IGF-1R antibodies 0035 and 0045 as reported herein (with one sided or both sided HHY-AAA mutation) shows in addition a faster clearance and shorter half-life in the serum as compared to the anti-IGF-1R antibody without HHY-AAA mutation (IGF-1R 0033).

[0806] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

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Lys	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val
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Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr 330 Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser 410 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala 420 425 Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys 440 <210> SEO ID NO 3 <211> LENGTH: 215 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEOUENCE: 3 Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr 25 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Asp Ala Ser Lys Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Lys Trp Pro Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ser Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val 185 Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys

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Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
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Ala Ile Ile Trp Phe Asp Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val
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Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Phe Cys
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Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
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Ala Ile Ile Trp Phe Asp Gly Ser Ser Lys Tyr Tyr Gly Asp Ser Val_{50}
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr 65 70 75 80
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Tyr Asp Ala Ser Lys Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
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		EQUEN			o sar) Lens	•								
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Lys	Arg	Leu	Glu 20	Asn	Сув	Thr	Val	Ile 25	Glu	Gly	Tyr	Leu	His 30	Ile	Leu
Leu	Ile	Ser 35	ГЛа	Ala	Glu	Asp	Tyr 40	Arg	Ser	Tyr	Arg	Phe 45	Pro	ГЛа	Leu
Thr	Val 50	Ile	Thr	Glu	Tyr	Leu 55	Leu	Leu	Phe	Arg	Val 60	Ala	Gly	Leu	Glu
Ser 65	Leu	Gly	Asp	Leu	Phe 70	Pro	Asn	Leu	Thr	Val 75	Ile	Arg	Gly	Trp	80 FÀa
Leu	Phe	Tyr	Asn	Tyr 85	Ala	Leu	Val	Ile	Phe 90	Glu	Met	Thr	Asn	Leu 95	Lys
Asp	Ile	Gly	Leu 100	Tyr	Asn	Leu	Arg	Asn 105	Ile	Thr	Arg	Gly	Ala 110	Ile	Arg
Ile	Glu	Lys 115	Asn	Ala	Asp	Leu	Cys 120	Tyr	Leu	Ser	Thr	Val 125	Asp	Trp	Ser
Leu	Ile 130	Leu	Asp	Ala	Val	Ser 135	Asn	Asn	Tyr	Ile	Val 140	Gly	Asn	Lys	Pro
Pro 145	Lys	Glu	Сув	Gly	Asp 150	Leu	Cys	Pro	Gly	Thr 155	Met	Glu	Glu	Lys	Pro 160
Met	Cys	Glu	Lys	Thr 165	Thr	Ile	Asn	Asn	Glu 170	Tyr	Asn	Tyr	Arg	Сув 175	Trp
Thr	Thr	Asn	Arg 180	Сув	Gln	Lys	Met	Сув 185	Pro	Ser	Thr	CÀa	Gly 190	Lys	Arg
Ala	Cys	Thr 195	Glu	Asn	Asn	Glu	Сув 200	Cys	His	Pro	Glu	Сув 205	Leu	Gly	Ser
CÀa	Ser 210	Ala	Pro	Asp	Asn	Asp 215	Thr	Ala	Cys	Val	Ala 220	CAa	Arg	His	Tyr
Tyr 225	Tyr	Ala	Gly	Val	Сув 230	Val	Pro	Ala	CÀa	Pro 235	Pro	Asn	Thr	Tyr	Arg 240
Phe	Glu	Gly	Trp	Arg 245	Cys	Val	Asp	Arg	Asp 250	Phe	Cys	Ala	Asn	Ile 255	Leu
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CÀa	Met	Gln 275	Glu	CAa	Pro	Ser	Gly 280	Phe	Ile	Arg	Asn	Gly 285	Ser	Gln	Ser
Met	Tyr 290	Cys	Ile	Pro	Cys	Glu 295	Gly	Pro	Cys	Pro	Lys 300	Val	Сув	Glu	Glu
Glu 305	Lys	Lys	Thr	Lys	Thr 310	Ile	Asp	Ser	Val	Thr 315	Ser	Ala	Gln	Met	Leu 320
Gln	Gly	Cys	Thr	Ile 325	Phe	Lys	Gly	Asn	Leu 330	Leu	Ile	Asn	Ile	Arg 335	Arg
Gly	Asn	Asn	Ile 340	Ala	Ser	Glu	Leu	Glu 345	Asn	Phe	Met	Gly	Leu 350	Ile	Glu
Val	Val	Thr	Gly	Tyr	Val	Lys	Ile	Arg	His	Ser	His	Ala	Leu	Val	Ser

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Leu	Trp	Asp	Trp	Asp 405	His	Arg	Asn	Leu	Thr 410	Ile	ГÀа	Ala	Gly	Lys 415	Met
Tyr	Phe	Ala	Phe 420	Asn	Pro	ГЛа	Leu	Cys 425	Val	Ser	Glu	Ile	Tyr 430	Arg	Met
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Thr	Arg 450	Asn	Asn	Gly	Glu	Arg 455	Ala	Ser	Сув	Glu	Ser 460	Asp	Val	Leu	His
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Arg	Tyr	Arg	Pro	Pro 485	Asp	Tyr	Arg	Asp	Leu 490	Ile	Ser	Phe	Thr	Val 495	Tyr
Tyr	Lys	Glu	Ala 500	Pro	Phe	Lys	Asn	Val 505	Thr	Glu	Tyr	Asp	Gly 510	Gln	Asp
Ala	Càa	Gly 515	Ser	Asn	Ser	Trp	Asn 520	Met	Val	Asp	Val	Asp 525	Leu	Pro	Pro
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Thr 545	Gln	Tyr	Ala	Val	Tyr 550	Val	Lys	Ala	Val	Thr 555	Leu	Thr	Met	Val	Glu 560
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Ser	Ser	Ser 595	Gln	Leu	Ile	Val	Lys 600	Trp	Asn	Pro	Pro	Ser 605	Leu	Pro	Asn
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Pro	Lys	Thr 675	Glu	Ala	Glu	Lys	Gln 680	Ala	Glu	Lys	Glu	Glu 685	Ala	Glu	Tyr
Arg	Lys 690	Val	Phe	Glu	Asn	Phe 695	Leu	His	Asn	Ser	Ile 700	Phe	Val	Pro	Arg
Pro 705	Glu	Arg	Lys	Arg	Arg 710	Asp	Val	Met	Gln	Val 715	Ala	Asn	Thr	Thr	Met 720
Ser	Ser	Arg	Ser	Arg 725	Asn	Thr	Thr	Ala	Ala 730	Asp	Thr	Tyr	Asn	Ile 735	Thr
Asp	Pro	Glu	Glu 740	Leu	Glu	Thr	Glu	Tyr 745	Pro	Phe	Phe	Glu	Ser 750	Arg	Val
Asp	Asn	Lys 755	Glu	Arg	Thr	Val	Ile 760	Ser	Asn	Leu	Arg	Pro 765	Phe	Thr	Leu

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795 Ala Asp Asp Ile Pro Gly Pro Val Thr Trp Glu Pro Arg Pro Glu As Sto Ser Ile Phe Leu Lys Trp Pro Glu Pro Glu Asn Pro Arg Pro Glu Asp Pro Val Asp Glu Glu Asp Glu Glu Asp Glu Glu Glu Asp Glu Glu Glu Asp Glu Glu Glu Glu Asp Glu	Tyr		Ile	Asp	Ile	His		Cys	Asn	His	Glu		Glu	Lys	Leu	Gly
Ser Ile Phe Leu Lys Trp Pro Glu Pro Glu Asn Pro Asn Gly Leu Ile Ile Ile Lys Trp Roy Gly Ser Gln Val Glu Asp Gln Arg Gls Ses Ses Arg Gln Glu Try Arg Lys Tyr Gly Gly Ala Lys Leu Arg Ses Arg Gln Glu Tyr Arg Lys Tyr Gly Gly Ala Lys Leu Arg Ass Asp Gly Asn Tyr Thr Ala Arg Ile Gln Ala Thr Ser Leu Ses Ser Gly Asn Gly Ser Trp Thr Asp Pro Val Phe Phe Tyr Val Gln Ala Arg Ser Gly Asn Gly Ser Trp Thr Asp Pro Val Phe Phe Tyr Val Gln Ala Val Leu Leu Ile Val Gly Gly Leu Val Ile Met Leu Tyr Val Phe Ser Arg Leu Gly Asn Gly Val Leu Tyr Arg Ser Val Asn Pro Glu Tyr Phe Ser Ala Ala Asp Val Tyr Val Pro Arg Ser Val Asn Pro Glu Tyr Phe Ser Ala Ala Asp Val Tyr Val Pro Arg Ser Arg Gly Val Ala Lys Gly Val Val Ser Ser Phe Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val Ser Ser Phe Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val Ser Ser Phe Gly Arg His His Val Val Arg Ser Val Asp Glu Pro Glu Thr Arg Val Ala Ile Lys Thr Val Asp Glu Asp Ser Tyr Leu Asp Glu Asp Asp Glu Pro Thr Leu Val Ile Met Glu Leu Gly Val 1005 10030 10035		Ser	Ala	Ser	Asn		Val	Phe	Ala	Arg		Met	Pro	Ala	Glu	Gly 800
See	Ala	Asp	Asp	Ile		Gly	Pro	Val	Thr	_	Glu	Pro	Arg	Pro		
Sa5	Ser	Ile	Phe		Lys	Trp	Pro	Glu		Glu	Asn	Pro	Asn			Ile
## S50	Leu	Met		Glu	Ile	Lys	Tyr		Ser	Gln	Val	Glu			Arg	Glu
865 870 875 88 Ser Gly Asn Gly Ser Trp Thr Asp Pro Val Phe Phe Tyr Val Gln Ass 885 890 New Phe Phe Tyr Val Gln Ass 895 New Phe Phe Tyr Val Phe Phe Phe Phe Tyr Val Phe Phe Phe Phe Phe Tyr Val Phe Phe Phe Tyr Val Phe	Сув		Ser	Arg	Gln	Glu		Arg	Lys	Tyr	Gly		Ala	Lys	Leu	Asn
Lys Thr Gly Tyr Glu Asn Phe IIe His Leu IIe IIe Ala Leu Pro Very 900 Ala Val Leu Leu IIe Val Gly Gly Leu Val IIe Met Leu Tyr Val Phe 915 His Arg Lys Arg Asn Asn Ser Arg Leu Gly Asn Gly Val Leu Tyr Al 930 Ser Val Asn Pro Glu Tyr Phe Ser Ala Ala Asp Val Tyr Val Pro 945 Glu Trp Glu Val Ala Arg Glu Lys IIe Thr Met Ser Arg Glu Leu Gly 965 Glu Trp Glu Val Ala Arg Glu Lys IIe Thr Met Ser Arg Glu Leu Gly 970 Gln Gly Ser Phe Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val 9980 Lys Asp Glu Pro Glu Thr Arg Val Ala IIe Lys Thr Val Asn Glu 995 Ala Ser Met Arg Glu Arg IIe Glu Phe Leu Asn Glu Ala Ser Val 1015 Ala Ser Gln Gly Gln Pro Thr Leu Val IIe Met Glu Leu Gly Val 1025 Arg Gly Asp Leu Lys Ser Tyr Leu Arg Ser Leu Arg Pro Glu Met 1045 Arg Gly Asp Leu Lys Ser Tyr Leu Arg Ser Leu Arg Pro Glu Met 1070 Gln Asn Asn Pro Val Leu Ala Pro Pro Ser Leu Ser Lys Met IIe 1070 Asn Lys Phe Val His Arg Asp Leu Ala Apg Gly Met Ala Tyr Leu Asn Ala 1085 Asn Lys Phe Val His Arg Asp Leu Ala Apg Phe Gly Met Val 1005 Ala Glu Asp Phe Thr Val Lys IIe Gly Asp Phe Gly Met Thr Arg 1120 Asp IIe Tyr Glu Thr Asp Tyr Tyr Arg Lys Gly Gly Lys Gly Leu Leu Apg IIe Tyr Glu Thr Arg 1120		Leu	Asn	Pro	Gly		Tyr	Thr	Ala	Arg		Gln	Ala	Thr	Ser	Leu 880
Ala Val Leu Leu Ile Val Gly Gly Leu Val Ile Met Leu Tyr Val Propriet 915	Ser	Gly	Asn	Gly		Trp	Thr	Asp	Pro		Phe	Phe	Tyr	Val		Ala
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930 935 940 Ser Val Asn Pro Glu Tyr Phe Ser Ala Ala Asp Val Tyr Val Pro Asp 955 955 965 Glu Trp Glu Val Ala Arg Glu Lys Ile Thr Met Ser Arg Glu Leu Gly Val Pro Asp 965 970 Gln Gly Ser Phe Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val 980 985 990 Lys Asp Glu Pro Glu Thr Arg Val Ala Ile Lys Thr Val Asn Glu 1005 Ala Ser Met Arg Glu Arg Ile Glu Phe Leu Asn Glu Ala Ser Val 1010 Met Lys Glu Phe Asn Cys His His Val Val Arg Leu Leu Gly Val 1025 Val Ser Gln Gly Gln Pro Thr Leu Val Ile Met Glu Leu Met Thr 1040 Arg Gly Asp Leu Lys Ser Tyr Leu Arg Ser Leu Arg Pro Glu Met 1055 Glu Asn Asn Pro Val Leu Ala Pro Pro Ser Leu Ser Lys Met Ile 1070 Gln Met Ala Gly Glu Ile Ala Asp Gly Met Ala Tyr Leu Asn Ala 1085 Asn Lys Phe Val His Arg Asp Leu Ala Ala Arg Asn Cys Met Val 1100 Ala Glu Asp Phe Thr Val Lys Ile Gly Asp Phe Gly Met Thr Arg 1115 Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys Gly Gly Lys Gly Leu Arg Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys Gly Gly Lys Gly Leu Arg Ile Incompany In	Ala	Val		Leu	Ile	Val	Gly		Leu	Val	Ile	Met			Val	Phe
945 950 955 960 Glu Trp Glu Val Ala Arg Glu Lys Ile Thr Met Ser Arg Glu Leu Gly Poppo Ser Phe Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val Poppo Ser Phe Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val Poppo Ser Phe Gly Met Val Tyr Glu Pro Glu Thr Arg Val Ala Ile Lys Thr Val Asn Glu Poppo Ser Met Arg Glu Arg Ile Glu Phe Leu Asn Glu Ala Ser Val 1010 Met Lys Glu Phe Asn Cys His His Val Val Arg Leu Leu Gly Val 1025 Val Ser Gln Gly Gln Pro Thr Leu Val Ile Met Glu Leu Met Thr 1040 Arg Gly Asp Leu Lys Ser Tyr Leu Arg Ser Leu Arg Pro Glu Met 1055 Glu Asn Asn Pro Val Leu Ala Pro Pro Ser Leu Ser Lys Met Ile 1070 Gln Met Ala Gly Glu Ile Ala Asp Gly Met Ala Tyr Leu Asn Ala 1085 Asn Lys Phe Val His Arg Asp Leu Ala Ala Arg Asn Cys Met Val 1100 Ala Glu Asp Phe Thr Val Lys Ile Gly Asp Phe Gly Met Thr Arg 1115 Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys Gly Gly Lys Gly Leu	His		Lys	Arg	Asn	Asn		Arg	Leu	Gly	Asn			Leu	Tyr	Ala
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Lys Asp Glu Pro Glu Thr Arg Val Ala Ile Lys Thr Val Asn Glu Ala Ser Met Arg Glu Arg Ile Glu Phe Leu Asn Glu Ala Ser Val Met Lys Glu Phe Asn Cys His His Val Val Arg Leu Glu Ala Ser Val Val Ser Glu Phe Asn Cys His His Val Val Arg Leu Glu Leu Glu Leu Met Thr Leu Val Ile Met Thr Leu Arg Ser Leu Met Thr Thr Leu Val Ile Ala Arg Pro Glu Met Thr Ala Ile Ala Arg Fro Pro P	Glu	Trp	Glu	Val		Arg	Glu	Lys	Ile		Met	Ser	Arg	Glu		
Ala Ser Met Arg Glu Arg Ile Glu Phe Leu Asn Glu Ala Ser Val 1010 Met Lys Glu Phe Asn Cys His His Val Val Arg Leu Leu Gly Val 1025 Val Ser Gln Gly Gln Pro Thr Leu Val Ile Met Glu Leu Met Thr 1040 Arg Gly Asp Leu Lys Ser Tyr Leu Arg Ser Leu Arg Pro Glu Met 1055 Glu Asn Asn Pro Val Leu Ala Pro Pro Ser Leu Ser Lys Met Ile 1070 Gln Met Ala Gly Glu Ile Ala Asp Gly Met Ala Tyr Leu Asn Ala 1085 Asn Lys Phe Val His Arg Asp Leu Ala Ala Arg Asn Cys Met Val 1100 Ala Glu Asp Phe Thr Val Lys Ile Gly Asp Phe Gly Met Thr Arg 1115 Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys Gly Gly Lys Gly Leu	Gln	Gly	Ser		Gly	Met	Val	Tyr		Gly	Val	Ala	Lys			Val
1010 Met Lys Glu Phe Asn Cys His 1030 Wal Ser Gln Gly Gln Pro Thr Leu Val Ile Met Glu Leu Met Thr 1040 Arg Gly Asp Leu Lys Ser Tyr Leu Arg Ser Leu Arg Pro Glu Met 1055 Glu Asn Asn Pro Val Leu Ala 1075 Gln Met 1085 Ala Gly Glu Ile Ala Asp Gly Met Ala Tyr Leu Asn Ala 1085 Asn Lys Phe Val His Arg Asp Leu Ala Ala Arg Asn Cys Met Val 1100 Ala Glu Asp Phe Thr Val Lys Ile Gly Asp Phe Gly Met Thr Arg 1115 Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys Gly Gly Lys Gly Leu Ala Leu Arg India Indi	Lys	Asp		Pro	Glu	Thr	Arg			a Il	e Ly	s Th			sn G	lu Ala
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Asn Lys Phe Val His Arg Asp Leu Ala Ala Arg Asn Cys Met Val 1100 1105 1110 Ala Glu Asp Phe Thr Val Lys Ile Gly Asp Phe Gly Met Thr Arg 1115 1120 1125 Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys Gly Gly Lys Gly Leu	Glu			n Pro	Val	l Leu			ro P	ro S	er L			Lys	Met	Ile
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	Ala		_	Phe	e Thi	r Val	_		le G	ly A	sp Pl		-	Met	Thr	Arg
	Asp		_	Glu	ı Thi	r Asp	_	_	yr A	rg L	ys G	-	_	Lys	Gly	Leu
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Glu	Ile 1175		Thr	Leu	Ala	Glu 118		n Pr	о Туз	Gln	Gly 1185	Leu	Ser	Asn
Glu	Gln 1190		Leu	. Arg	Phe	Val 119		t Gl	u Gly	/ Gly	Leu 1200		Asp	Lys
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Trp	Gln 1220	_	Asn	Pro	Lys	Met 122		g Pr	o Sei	? Phe	Leu 1230		Ile	Ile
Ser	Ser 1235		Lys	Glu	Glu	Met 124		u Pr	o Gly	/ Phe	Arg 1245	Glu	Val	Ser
Phe	Tyr 1250		Ser	Glu	Glu	Asn 125		s Le	u Pro	Glu	Pro 1260	Glu	Glu	Leu
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Lys	Ala 1295		Asn	. Gly	Pro	Gly 130		o Gl	y Val	l Leu	Val 1305		Arg	Ala
Ser	Phe 1310	_	Glu	. Arg	Gln	Pro 131	_	r Al	a His	Met	Asn 1320	Gly	Gly	Arg
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Ser	Thr		Gly 20	Gly	Thr	Ala								
Phe	_							Leu (25	Gly (Cys L	eu Vai	30 1 Lys	a Asr	Tyr
		Glu 35		Val	Thr	Val	:	25	_	_	eu Val ly Ala 45	30	_	_
Gly		35	Pro		Pro	Val	Ser '	25 Trp 2	Asn S	- Ser G	ly Ala 45 er Gly	30 a Lei	ı Thi	Ser
	Val 50	35 His	Pro Thr	Phe	Pro	Val Ala 55	Ser ' 40 Val :	25 Trp :	Asn S Gln S	Ser G Ser S	ly Ala 45 er Gly	30 a Lei	ı Thi	Ser Ser
Leu 65	Val 50 Ser	35 His Ser	Pro Thr Val	Phe Val	Pro Thr 70	Val Ala 55 Val	Ser '40 Val :	25 Trp : Leu (Ser :	Asn S	Ser G Ser S 6 Ser L	ly Ala 45 er Gly	30 a Let y Let y Thi	ı Thi ı Tyi r Glr	Ser Ser Thr
Leu 65 Tyr	Val 50 Ser	35 His Ser Cys	Pro Thr Val Asn	Phe Val Val 85	Pro Thr 70 Asn	Val Ala 55 Val His	Ser '40 Val : Pro : Lys :	Trp : Leu (Ser :	Asn S	Ser G Ser S 6 Ser L 75	ly Ala 45 er Gly 0	30 a Let Y Let Y Thi S Val	I Thi I Tyi r Gli l Asi 95	Ser Ser Thr 80
Leu 65 Tyr Lys	Val 50 Ser Ile Val	35 His Ser Cys	Pro Thr Val Asn Pro	Phe Val Val 85 Lys	Pro Thr 70 Asn	Val Ala 55 Val His Cys	Ser 40 Val : Pro : Lys :	Trp : Leu (Ser : Pro : Lys (Asn S Gln S Ser S Ser I	Ser G Ser L 75 Asn T	ly Ala 45 er Gly 0 eu Gly hr Lys	30 a Let Y Let Y Thi S Val S Pro 110	ı Thı ı Tyı r Glr l Ası 95	Ser Ser Thr 80 Lys
Leu 65 Tyr Lys Pro	Val 50 Ser Ile Val	35 His Ser Cys Glu Pro	Pro Thr Val Asn Pro 100 Glu	Phe Val Val 85 Lys Leu	Pro Thr 70 Asn Ser Leu Leu	Val Ala 55 Val His Cys	Ser 40 Val : Pro : Lys : Gly : Gly :	Trp : Leu (Ser : Pro : Lys Pro :	Ser : Ser : Thr I	Ser G Ser L 75 Asn T His T	ly Ala 45 er Gly 0 eu Gly hr Lys hr Cys	300 300 300 300 400 400 400 400 400 400	Thin Tyr Glr Asp 95	Ser Thr 80 Lys Cys
Leu 65 Tyr Lys Pro	Val 50 Ser Ile Val Ala Pro	35 His Ser Cys Glu Pro 115 Lys	Pro Thr Val Asn Pro 100 Glu Asp	Phe Val Val 85 Lys Leu Thr	Pro Thr 70 Asn Ser Leu	Val Ala 55 Val His Cys Gly Met 135	Ser 40 Val : Pro : Lys : Gly : 120 Ile :	Leu (Ser : Lys (105) Pro :	AAsn Ser	Ger See See See See Lessen T. See Seer Les	ly Ala 45 er Gly 0 eu Gly hr Ly: hr Cy: he Let 129	30 a Let Y Let Y Throw T	I Thing Type of The Type of Ty	Ser Thr 80 Lys Cys Pro
Leu 65 Tyr Lys Pro Lys Val 145	Val 50 Ser Ile Val Ala Pro 130 Val	35 His Ser Cys Glu Pro 115 Lys	Pro Thr Val Asn Pro 100 Glu Asp	Phe Val Val 85 Lys Leu Thr	Pro Thr 70 Asn Ser Leu Leu Ser 150	Val Ala 55 Val His Cys Gly Met 135 His	Ser 40 Val : Pro : Lys : Asp : 120 Ile :	ZSE CONTROL OF THE PROPERTY OF	Asn S Gln S Ser 1 Thr F Arg 5	Ser G Ser Ser L Ser L	ly Ala 45 er Gly 0 eu Gly hr Lys hr Cys he Leu 129 ro Glu	30 Let Value	Thing Type of the second of th	Ser Ser Thr 80 Lys Cys Cys Cys Trp 160 Glu

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu 185 His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly 215 Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu 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 Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe 280 285 Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn 295 Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr 310 315 Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys 325 <210> SEQ ID NO 13 <211> LENGTH: 327 <212> TYPE: PRT <213> ORGANISM: Homo sapiens <400> SEQUENCE: 13 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg 10 Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val 135 Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp 155 150 Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp 185 Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu

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195
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Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
  210 215
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
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Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
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Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
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Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
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Gln	Tyr	Gln 35	Val	Gln	His	Gly	Ser 40	Сув	Ser	Tyr	Thr	Phe 45	Leu	Leu	Pro
Glu	Met 50	Asp	Asn	Сув	Arg	Ser 55	Ser	Ser	Ser	Pro	Tyr 60	Val	Ser	Asn	Ala
Val 65	Gln	Arg	Asp	Ala	Pro 70	Leu	Glu	Tyr	Asp	Asp 75	Ser	Val	Gln	Arg	Leu 80
Gln	Val	Leu	Glu	Asn 85	Ile	Met	Glu	Asn	Asn 90	Thr	Gln	Trp	Leu	Met 95	Lys
Leu	Glu	Asn	Tyr 100	Ile	Gln	Asp	Asn	Met 105	Lys	Lys	Glu	Met	Val 110	Glu	Ile
Gln	Gln	Asn 115	Ala	Val	Gln	Asn	Gln 120	Thr	Ala	Val	Met	Ile 125	Glu	Ile	Gly
Thr	Asn 130	Leu	Leu	Asn	Gln	Thr 135	Ala	Glu	Gln	Thr	Arg 140	Lys	Leu	Thr	Asp
Val 145	Glu	Ala	Gln	Val	Leu 150	Asn	Gln	Thr	Thr	Arg 155	Leu	Glu	Leu	Gln	Leu 160
Leu	Glu	His	Ser	Leu 165	Ser	Thr	Asn	Lys	Leu 170	Glu	Lys	Gln	Ile	Leu 175	Asp
Gln	Thr	Ser	Glu 180	Ile	Asn	Lys	Leu	Gln 185	Asp	Lys	Asn	Ser	Phe 190	Leu	Glu
Lys	Lys	Val 195	Leu	Ala	Met	Glu	Asp 200	Lys	His	Ile	Ile	Gln 205	Leu	Gln	Ser
Ile	Lys 210	Glu	Glu	Lys	Asp	Gln 215	Leu	Gln	Val	Leu	Val 220	Ser	Lys	Gln	Asn
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Asn	Ser	Val	Leu	Gln 245	Lys	Gln	Gln	His	Asp 250	Leu	Met	Glu	Thr	Val 255	Asn
Asn	Leu	Leu	Thr 260	Met	Met	Ser	Thr	Ser 265	Asn	Ser	Ala	ГÀа	Asp 270	Pro	Thr
Val	Ala	Lys 275	Glu	Glu	Gln	Ile	Ser 280	Phe	Arg	Asp	Cys	Ala 285	Glu	Val	Phe
Lys	Ser 290	Gly	His	Thr	Thr	Asn 295	Gly	Ile	Tyr	Thr	Leu 300	Thr	Phe	Pro	Asn
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Tyr	Trp	Leu 355	Gly	Asn	Glu	Phe	Val 360	Ser	Gln	Leu	Thr	Asn 365	Gln	Gln	Arg
Tyr	Val 370	Leu	Lys	Ile	His	Leu 375	Lys	Asp	Trp	Glu	Gly 380	Asn	Glu	Ala	Tyr
Ser 385	Leu	Tyr	Glu	His	Phe 390	Tyr	Leu	Ser	Ser	Glu 395	Glu	Leu	Asn	Tyr	Arg 400
Ile	His	Leu	Lys	Gly 405	Leu	Thr	Gly	Thr	Ala 410	Gly	Lys	Ile	Ser	Ser 415	Ile
Ser	Gln	Pro	Gly	Asn	Asp	Phe	Ser	Thr	Lys	Asp	Gly	Asp	Asn	Asp	Lys

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Cys Ile	Сув 435	Lys	СЛа	Ser	Gln	Met 440	Leu	Thr	Gly	Gly	Trp 445	Trp	Phe	Asp
Ala Cys 450	Gly	Pro	Ser	Asn	Leu 455	Asn	Gly	Met	Tyr	Tyr 460	Pro	Gln	Arg	Gln
Asn Thr 465	Asn	Lys	Phe	Asn 470	Gly	Ile	Lys	Trp	Tyr 475	Tyr	Trp	Lys	Gly	Ser 480
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Tyr Asn	Arg 35	Ile	Gln	His	Gly	Gln 40	Cys	Ala	Tyr	Thr	Phe 45	Ile	Leu	Pro
Glu His 50	Asp	Gly	Asn	Cys	Arg 55	Glu	Ser	Thr	Thr	Asp	Gln	Tyr	Asn	Thr
Asn Ala 65	Leu	Gln	Arg	Asp 70	Ala	Pro	His	Val	Glu 75	Pro	Asp	Phe	Ser	Ser 80
Gln Lys	Leu	Gln	His 85	Leu	Glu	His	Val	Met 90	Glu	Asn	Tyr	Thr	Gln 95	Trp
Leu Gln	Lys	Leu 100	Glu	Asn	Tyr	Ile	Val 105	Glu	Asn	Met	ГÀЗ	Ser 110	Glu	Met
Ala Gln	115					120					125			
Glu Ile 130					135					140				
Leu Thr	_			150					155					160
Ile Gln			165					170					175	
Leu Leu		180					185					190		
Leu Leu	195					200					205			
Leu Asp 210					215	-				220				
Arg Gln 225	Thr	Tyr	Ile	11e 230	Gln	Glu	Leu	Glu	Lys 235	Gln	Leu	Asn	Arg	Ala 240
Thr Thr	Asn	Asn	Ser 245	Val	Leu	Gln	Lys	Gln 250	Gln	Leu	Glu	Leu	Met 255	Asp
Thr Val	His	Asn 260	Leu	Val	Asn	Leu	Сув 265	Thr	Lys	Glu	Gly	Val 270	Leu	Leu
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Phe Gln Arg	Gly 340	Trp	Lys	Glu	Tyr	Lys 345	Met	Gly	Phe	Gly	Asn 350	Pro	Ser
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Ser Leu Ile	Leu 420	His	Gly	Ala	Asp	Phe 425	Ser	Thr	Lys	Asp	Ala 430	Asp	Asn
Asp Asn Cys 435	Met	Cys	Lys	CAa	Ala 440	Leu	Met	Leu	Thr	Gly 445	Gly	Trp	Trp
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Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile 290 295 300

Lys 145	Val	Leu	Ile	Lys	Glu 150	Glu	Asp	Ala	Val	Ile 155	Tyr	ГÀа	Asn	Gly	Ser 160
Phe	Ile	His	Ser	Val 165	Pro	Arg	His	Glu	Val 170	Pro	Asp	Ile	Leu	Glu 175	Val
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Arg	Arg 210	Cys	Glu	Ala	Gln	Lys 215	Trp	Gly	Pro	Glu	Cys 220	Asn	His	Leu	Cys
Thr 225	Ala	Cys	Met	Asn	Asn 230	Gly	Val	Cys	His	Glu 235	Asp	Thr	Gly	Glu	Cys 240
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Cys	Ala 290	Thr	Gly	Trp	Lys	Gly 295	Leu	Gln	Cys	Asn	Glu 300	Ala	Cys	His	Pro
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Glu	Met	Cys	Asp	Arg 325	Phe	Gln	Gly	Cys	Leu 330	Сла	Ser	Pro	Gly	Trp 335	Gln
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Val	Glu	Lys 435	Pro	Phe	Asn	Ile	Ser 440	Val	Lys	Val	Leu	Pro 445	Lys	Pro	Leu
Asn	Ala 450	Pro	Asn	Val	Ile	Asp 455	Thr	Gly	His	Asn	Phe 460	Ala	Val	Ile	Asn
Ile 465	Ser	Ser	Glu	Pro	Tyr 470	Phe	Gly	Asp	Gly	Pro 475	Ile	Lys	Ser	Lys	Lys 480
Leu	Leu	Tyr	Lys	Pro 485	Val	Asn	His	Tyr	Glu 490	Ala	Trp	Gln	His	Ile 495	Gln
Val	Thr	Asn	Glu 500	Ile	Val	Thr	Leu	Asn 505	Tyr	Leu	Glu	Pro	Arg 510	Thr	Glu
Tyr	Glu	Leu 515	Cys	Val	Gln	Leu	Val 520	Arg	Arg	Gly	Glu	Gly 525	Gly	Glu	Gly
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Glu	Gln 610	Tyr	Val	Val	Arg	Ala 615	Arg	Val	Asn	Thr	Lys 620	Ala	Gln	Gly	Glu
Trp 625	Ser	Glu	Asp	Leu	Thr 630	Ala	Trp	Thr	Leu	Ser 635	Asp	Ile	Leu	Pro	Pro 640
Gln	Pro	Glu	Asn	Ile 645	Lys	Ile	Ser	Asn	Ile 650	Thr	His	Ser	Ser	Ala 655	Val
Ile	Ser	Trp	Thr 660	Ile	Leu	Asp	Gly	Tyr 665	Ser	Ile	Ser	Ser	Ile 670	Thr	Ile
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Ile	Lys 690	Asn	Ala	Thr	Ile	Thr 695	Gln	Tyr	Gln	Leu	Lys 700	Gly	Leu	Glu	Pro
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Pro	Val	Leu	Asp 820	Trp	Asn	Asp	Ile	Lys 825	Phe	Gln	Asp	Val	Ile 830	Gly	Glu
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Asp 865	His	Arg	Asp	Phe	Ala 870	Gly	Glu	Leu	Glu	Val 875	Leu	Cys	Lys	Leu	Gly 880
His	His	Pro	Asn	Ile 885	Ile	Asn	Leu	Leu	Gly 890	Ala	Сув	Glu	His	Arg 895	Gly
Tyr	Leu	Tyr	Leu 900	Ala	Ile	Glu	Tyr	Ala 905	Pro	His	Gly	Asn	Leu 910	Leu	Asp
Phe	Leu	Arg 915	Lys	Ser	Arg	Val	Leu 920	Glu	Thr	Asp	Pro	Ala 925	Phe	Ala	Ile
Ala	Asn 930	Ser	Thr	Ala	Ser	Thr 935	Leu	Ser	Ser	Gln	Gln 940	Leu	Leu	His	Phe
Ala 945	Ala	Asp	Val	Ala	Arg 950	Gly	Met	Asp	Tyr	Leu 955	Ser	Gln	Lys	Gln	Phe 960

Ile His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Gly Glu Asn Tyr Val Ala Lys Ile Ala Asp Phe Gly Leu Ser Arg Gly Gln Glu Val Tyr Val Lys Lys Thr Met Gly Arg Leu Pro Val Arg Trp Met Ala Ile Glu 1000 Ser Leu Asn Tyr Ser Val Tyr Thr Thr Asn Ser Asp Val Trp Ser Tyr Gly Val Leu Leu Trp Glu Ile Val Ser Leu Gly Gly Thr Pro Tyr Cys Gly Met Thr Cys Ala Glu Leu Tyr Glu Lys Leu Pro Gln 1045 Gly Tyr Arg Leu Glu Lys Pro Leu Asn Cys Asp Asp Glu Val Tyr 1060 Asp Leu Met Arg Gln Cys Trp Arg Glu Lys Pro Tyr Glu Arg Pro 1075 1080 Ser Phe Ala Gln Ile Leu Val Ser Leu Asn Arg Met Leu Glu Glu 1095 1085 1090 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 1120 <210> SEQ ID NO 34 <211> LENGTH: 453 <212> TYPE: PRT <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: Heavy chain 1 of <VEGF-ANG-2> CrossMAb IgG1 with AAA mutations (VEGFang2-0012) <400> SEQUENCE: 34 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr 25 Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Tyr Pro Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val 105 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly 120 Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe

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Pro	Ala	Val	Leu 180	Gln	Ser	Ser	Gly	Leu 185		Ser	Leu	Ser	Ser 190	Val	Val	
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Ser 225	Cys	Asp	Lys	Thr	His 230	Thr	Cys	Pro	Pro	Cys 235	Pro	Ala	Pro	Glu	Leu 240	
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Val	Ser 370	Leu	Trp	CAa	Leu	Val 375	Lys	Gly	Phe	Tyr	Pro 380	Ser	Asp	Ile	Ala	
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Ser	Thr	Tyr 195	Ser	Leu	Ser	Ser	Thr 200	Leu	Thr	Leu	Ser	Lys 205	Ala	Asp	Tyr
Glu	Lys 210	His	Lys	Val	Tyr	Ala 215	Cys	Glu	Val	Thr	His 220	Gln	Gly	Leu	Ser
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Thr	СЛа	Pro	Pro	Cys 245	Pro	Ala	Pro	Glu	Leu 250	Leu	Gly	Gly	Pro	Ser 255	Val
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Pro	Glu	Val 275	Thr	CAa	Val	Val	Val 280	Asp	Val	Ser	His	Glu 285	Asp	Pro	Glu
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Thr 305	Lys	Pro	Arg	Glu	Glu 310	Gln	Tyr	Asn	Ser	Thr 315	Tyr	Arg	Val	Val	Ser 320
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Cys	Lys	Val	Ser 340	Asn	Lys	Ala	Leu	Pro 345	Ala	Pro	Ile	Glu	Lys 350	Thr	Ile
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Pro	Ser 370	Arg	Asp	Glu	Leu	Thr 375	Lys	Asn	Gln	Val	Ser 380	Leu	Ser	Cys	Ala
Val 385	Lys	Gly	Phe	Tyr	Pro 390	Ser	Asp	Ile	Ala	Val 395	Glu	Trp	Glu	Ser	Asn 400
Gly	Gln	Pro	Glu	Asn 405	Asn	Tyr	Lys	Thr	Thr 410	Pro	Pro	Val	Leu	Asp 415	Ser
Asp	Gly	Ser	Phe 420	Phe	Leu	Val	Ser	Lys 425	Leu	Thr	Val	Asp	Lys 430	Ser	Arg
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Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
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Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
                   105
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
     115 120
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
                     135
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
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Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
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Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
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His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
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Asp	Glu	Ala	Asp	Tyr 85	Tyr	CÀa	Gln	Val	Trp 90	Asp	Ser	Ser	Ser	Asp 95	His
Trp	Val	Phe	Gly 100	Gly	Gly	Thr	Lys	Leu 105	Thr	Val	Leu	Ser	Ser 110	Ala	Ser
Thr	Lys	Gly 115	Pro	Ser	Val	Phe	Pro 120	Leu	Ala	Pro	Ser	Ser 125	Lys	Ser	Thr
Ser	Gly 130	Gly	Thr	Ala	Ala	Leu 135	Gly	Cys	Leu	Val	Lys 140	Asp	Tyr	Phe	Pro
Glu 145	Pro	Val	Thr	Val	Ser 150	Trp	Asn	Ser	Gly	Ala 155	Leu	Thr	Ser	Gly	Val 160
His	Thr	Phe	Pro	Ala 165	Val	Leu	Gln	Ser	Ser 170	Gly	Leu	Tyr	Ser	Leu 175	Ser
Ser	Val	Val	Thr 180	Val	Pro	Ser	Ser	Ser 185	Leu	Gly	Thr	Gln	Thr 190	Tyr	Ile
Cys	Asn	Val 195	Asn	His	Lys	Pro	Ser 200	Asn	Thr	Lys	Val	Asp 205	Lys	Lys	Val
Glu	Pro 210	Lys	Ser	CÀa											
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Pro	Ile	Glu	Lys 340	Thr	Ile	Ser	Lys	Ala 345	Lys	Gly	Gln	Pro	Arg 350	Glu	Pro
Gln	Val	Tyr 355	Thr	Leu	Pro	Pro	360	Arg	Asp	Glu	Leu	Thr 365	ГÀв	Asn	Gln
Val	Ser 370	Leu	Trp	CAa	Leu	Val 375	Lys	Gly	Phe	Tyr	Pro 380	Ser	Asp	Ile	Ala
Val 385	Glu	Trp	Glu	Ser	Asn 390	Gly	Gln	Pro	Glu	Asn 395	Asn	Tyr	ГÀЗ	Thr	Thr 400
Pro	Pro	Val	Leu	Asp 405	Ser	Asp	Gly	Ser	Phe 410	Phe	Leu	Tyr	Ser	Lys 415	Leu
Thr	Val	Asp	Lys 420	Ser	Arg	Trp	Gln	Gln 425	Gly	Asn	Val	Phe	Ser 430	Cys	Ser
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Tyr	Met	His 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Gln	Gly	Leu 45	Glu	Trp	Met

Gly	Trp 50	Ile	Asn	Pro	Asn	Ser 55	Gly	Gly	Thr	Asn	Tyr 60	Ala	Gln	ГÀа	Phe
Gln 65	Gly	Arg	Val	Thr	Met 70	Thr	Arg	Asp	Thr	Ser 75	Ile	Ser	Thr	Ala	Tyr 80
Met	Glu	Leu	Ser	Arg 85	Leu	Arg	Ser	Asp	Asp 90	Thr	Ala	Val	Tyr	Tyr 95	CÀa
Ala	Arg	Ser	Pro 100	Asn	Pro	Tyr	Tyr	Tyr 105	Asp	Ser	Ser	Gly	Tyr 110	Tyr	Tyr
Pro	Gly	Ala 115	Phe	Asp	Ile	Trp	Gly 120	Gln	Gly	Thr	Met	Val 125	Thr	Val	Ser
Ser	Ala 130	Ser	Val	Ala	Ala	Pro 135	Ser	Val	Phe	Ile	Phe 140	Pro	Pro	Ser	Asp
Glu 145	Gln	Leu	Lys	Ser	Gly 150	Thr	Ala	Ser	Val	Val 155	Cys	Leu	Leu	Asn	Asn 160
Phe	Tyr	Pro	Arg	Glu 165	Ala	Lys	Val	Gln	Trp 170	Lys	Val	Asp	Asn	Ala 175	Leu
Gln	Ser	Gly	Asn 180	Ser	Gln	Glu	Ser	Val 185	Thr	Glu	Gln	Asp	Ser 190	Lys	Asp
Ser	Thr	Tyr 195	Ser	Leu	Ser	Ser	Thr 200	Leu	Thr	Leu	Ser	Lys 205	Ala	Asp	Tyr
Glu	Lys 210	His	Lys	Val	Tyr	Ala 215	Сув	Glu	Val	Thr	His 220	Gln	Gly	Leu	Ser
Ser 225	Pro	Val	Thr	Lys	Ser 230	Phe	Asn	Arg	Gly	Glu 235	CAa	Asp	Lys	Thr	His 240
Thr	Cys	Pro	Pro	Cys 245	Pro	Ala	Pro	Glu	Ala 250	Ala	Gly	Gly	Pro	Ser 255	Val
Phe	Leu	Phe	Pro 260	Pro	rys	Pro	Lys	Asp 265	Thr	Leu	Met	Ala	Ser 270	Arg	Thr
Pro	Glu	Val 275	Thr	Сув	Val	Val	Val 280	Asp	Val	Ser	His	Glu 285	Asp	Pro	Glu
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CAa	Lys	Val	Ser 340	Asn	Lys	Ala	Leu	Gly 345	Ala	Pro	Ile	Glu	Lys 350	Thr	Ile
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Pro	Ser 370	Arg	Asp	Glu	Leu	Thr 375	Lys	Asn	Gln	Val	Ser 380	Leu	Ser	CÀa	Ala
Val 385	Lys	Gly	Phe	Tyr	Pro 390	Ser	Asp	Ile	Ala	Val 395	Glu	Trp	Glu	Ser	Asn 400
Gly	Gln	Pro	Glu	Asn 405	Asn	Tyr	Lys	Thr	Thr 410	Pro	Pro	Val	Leu	Asp 415	Ser
Asp	Gly	Ser	Phe 420	Phe	Leu	Val	Ser	Lys 425	Leu	Thr	Val	Asp	Lys 430	Ser	Arg
Trp	Gln	Gln 435	Gly	Asn	Val	Phe	Ser 440	Cys	Ser	Val	Met	His	Glu	Ala	Leu
His	Asn	Ala	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	

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Leu Asn Trj 35	o Tyr Gln	Gln Lys	Pro Gly 40	Lys Ala	Pro Lys V	al Leu Ile
Tyr Phe Th	r Ser Ser	Leu His 55	Ser Gly	Val Pro	Ser Arg P	he Ser Gly
Ser Gly Se	r Gly Thr	Asp Phe	Thr Leu	Thr Ile	Ser Ser L	eu Gln Pro 80
Glu Asp Ph	e Ala Thr 85	Tyr Tyr	Cys Gln	Gln Tyr 90	Ser Thr V	al Pro Trp 95
Thr Phe Gl	y Gln Gly 100	Thr Lys	Val Glu 105			al Ala Ala 10
Pro Ser Vai		Phe Pro	Pro Ser 120	Asp Glu	Gln Leu L 125	ys Ser Gly
Thr Ala Se	r Val Val	Cys Leu 135	Leu Asn	Asn Phe	Tyr Pro A	rg Glu Ala
Lys Val Gl	n Trp Lys	Val Asp 150	Asn Ala	Leu Gln 155	Ser Gly A	sn Ser Gln 160
Glu Ser Va	l Thr Glu 165	_	Ser Lys	Asp Ser 170	Thr Tyr S	er Leu Ser 175
Ser Thr Le	ı Thr Leu 180	Ser Lys	Ala Asp 185			ys Val Tyr 90
Ala Cys Gli		His Gln	Gly Leu 200	. Ser Ser	Pro Val T	hr Lys Ser
Phe Asn Arg	g Gly Glu	Сув				
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Thr Ala Ar	g Ile Thr 20	Cys Gly	Gly Asn 25	Asn Ile	Gly Ser L	•
His Trp Ty:	r Gln Gln	Lys Pro	Gly Gln 40	Ala Pro	Val Leu V	al Val Tyr
Asp Asp Se	r Asp Arg	Pro Ser	Gly Ile	Pro Glu	Arg Phe S	er Gly Ser

																_
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Thr	Lys	Gly 115	Pro	Ser	Val	Phe	Pro 120	Leu	Ala	Pro	Ser	Ser 125	Lys	Ser	Thr	
Ser	Gly 130	Gly	Thr	Ala	Ala	Leu 135	Gly	Cya	Leu	Val	Lys 140	Asp	Tyr	Phe	Pro	
Glu 145	Pro	Val	Thr	Val	Ser 150	Trp	Asn	Ser	Gly	Ala 155	Leu	Thr	Ser	Gly	Val 160	
His	Thr	Phe	Pro	Ala 165	Val	Leu	Gln	Ser	Ser 170	Gly	Leu	Tyr	Ser	Leu 175	Ser	
Ser	Val	Val	Thr 180	Val	Pro	Ser	Ser	Ser 185	Leu	Gly	Thr	Gln	Thr 190	Tyr	Ile	
CAa	Asn	Val 195	Asn	His	Lys	Pro	Ser 200	Asn	Thr	Lys	Val	Asp 205	Lys	ГÀв	Val	
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	D> SI	EQUEI	ICE :	42	ions	3 8	and v	vith	SPLI	E mut	tatio	ons				
		EQUEI	ICE :	42	cions	3 8	and v	vith	SPLI	E mut	tatio	ons				
Glu 1	D> SI	EQUEN Gln	ICE : Leu	42 Val 5	ions Glu	Ser	and v	vith Gly	SPLE Gly 10	E mut Leu	val	ons Gln	Pro	Gly 15	Gly	
Glu 1 Ser	D> SI Val	EQUEN Gln Arg	ICE: Leu Leu 20	42 Val 5 Ser	Glu Cys	Ser Ala	Gly Ala	Gly Ser 25	Gly 10	E mut Leu Tyr	Val Asp	Gln Phe	Pro Thr 30	Gly 15 His	Gly Tyr	
Glu 1 Ser Gly	D> SI Val Leu	Gln Arg Asn 35	Leu Leu Leu 20 Trp	42 Val 5 Ser	Glu Cys Arg	Ser Ala	Gly Ala Ala 40	Gly Ser 25 Pro	Gly 10 Gly Gly	Leu Tyr Lys	Val Asp Gly	Gln Phe Leu 45	Pro Thr 30 Glu	Gly 15 His	Gly Tyr Val	
Glu 1 Ser Gly	D> SI Val Leu Met	Gln Arg Asn 35 Ile	Leu Leu 20 Trp Asn	42 Val 5 Ser Val Thr	Glu Cys Arg	Ser Ala Gln Thr	Gly Ala Ala 40 Gly	Gly Ser 25 Pro Glu	Gly 10 Gly Gly	Leu Tyr Lys	Val Asp Gly Tyr	Gln Phe Leu 45 Ala	Pro Thr 30 Glu Ala	Gly 15 His Trp	Gly Tyr Val Phe	
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Glu 1 Ser Gly Gly Lys 65 Leu)> SH Val Leu Met Trp 50 Arg	Gln Arg Asn 35 Ile Arg Met	Leu Leu 20 Trp Asn Phe Asn Pro 100	Val Ser Val Thr Thr Ser 85	Glu Cys Arg Tyr Phe 70 Leu	Ser Ala Gln Thr 55 Ser Arg	Gly Ala Ala 40 Gly Leu Ala	Gly Ser 25 Pro Glu Asp Glu Thr 105	Gly 10 Gly Pro Thr Asp 90 Ser	Leu Tyr Lys Thr Ser 75 Thr	Val Asp Gly Tyr 60 Lys Ala	Gln Phe Leu 45 Ala Ser Val	Pro Thr 30 Glu Ala Thr Tyr Phe 110	Gly 15 His Trp Asp Ala Tyr 95	Gly Tyr Val Phe Tyr 80 Cys	
Glu 1 Ser Gly Gly Lys 65 Leu Ala	O> SF Val Leu Met Trp 50 Arg Gln	EQUEN Gln Arg Asn 35 Ile Arg Met Tyr	Leu Leu 20 Trp Asn Phe Asn Gly	42 Val 5 Ser Val Thr Thr Thr Thr	Glu Cys Arg Tyr Phe 70 Leu Tyr	Ser Ala Gln Thr 55 Ser Arg Tyr	Gly Ala Ala 40 Gly Leu Ala Gly Thr	Gly Ser 25 Pro Glu Asp Glu Thr 105 Val	Gly 10 Gly Pro Thr Asp 90 Ser	Leu Tyr Lys Thr Ser 75 Thr His	Val Asp Gly Tyr 60 Lys Ala Trp	Gln Phe Leu 45 Ala Ser Val Tyr Ser 125	Pro Thr 30 Glu Ala Thr Tyr Phe 110 Thr	Gly 15 His Trp Asp Ala Tyr 95 Asp	Gly Tyr Val Phe Tyr 80 Cys Val Gly	
Glu 1 Ser Gly Gly Lys 65 Leu Ala Trp	O> SI Val Leu Met Trp 50 Arg Gln Lys Gly Ser	EQUENT Gln Arg Asn 35 Ile Arg Met Tyr Gln 115 Val	Leu Leu 20 Trp Asn Phe Asn Gly Phe	Val 5 Ser Val Thr Thr Thr Pro	Glu Cys Arg Tyr Phe 70 Leu Tyr Leu Leu	Ser Ala Gln Thr 55 Ser Arg Tyr Val	Gly Ala Ala 40 Gly Leu Ala Gly Thr 120 Pro	Gly Ser 25 Pro Glu Asp Glu Thr 105 Val	Gly 10 Gly Pro Thr Asp 90 Ser Ser Ser	Leu Tyr Lys Thr Ser Thr His	Val Asp Gly Tyr 60 Lys Ala Trp Ala Ser 140	Gln Phe Leu 45 Ala Ser Val Tyr Ser 125 Thr	Pro Thr 30 Glu Ala Thr Tyr Phe 110 Thr	Gly 15 His Trp Asp Ala Tyr 95 Asp Lys	Gly Tyr Val Phe Tyr 80 Cys Val Gly Ser	

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Asp	His 210	Lys	Pro	Ser	Asn	Thr 215	Lys	Val	Asp	Lys	Arg 220	Val	Glu	Ser	Lys	
Tyr 225	Gly	Pro	Pro	Сла	Pro 230	Pro	Cys	Pro	Ala	Pro 235	Glu	Phe	Glu	Gly	Gly 240	
Pro	Ser	Val	Phe	Leu 245	Phe	Pro	Pro	Lys	Pro 250	ГÀз	Asp	Thr	Leu	Met 255	Ala	
Ser	Arg	Thr	Pro 260	Glu	Val	Thr	Cys	Val 265	Val	Val	Asp	Val	Ser 270	Gln	Glu	
Asp	Pro	Glu 275	Val	Gln	Phe	Asn	Trp 280	Tyr	Val	Asp	Gly	Val 285	Glu	Val	His	
Asn	Ala 290	Lys	Thr	Lys	Pro	Arg 295	Glu	Glu	Gln	Phe	Asn 300	Ser	Thr	Tyr	Arg	
Val 305	Val	Ser	Val	Leu	Thr 310	Val	Leu	Ala	Gln	Asp 315	Trp	Leu	Asn	Gly	Lys 320	
Glu	Tyr	Lys	Cya	Lys 325	Val	Ser	Asn	Lys	Gly 330	Leu	Pro	Ser	Ser	Ile 335	Glu	
Lys	Thr	Ile	Ser 340	Lys	Ala	Lys	Gly	Gln 345	Pro	Arg	Glu	Pro	Gln 350	Val	Cys	
Thr	Leu	Pro 355	Pro	Ser	Gln	Glu	Glu 360	Met	Thr	Lys	Asn	Gln 365	Val	Ser	Leu	
Ser	Cys 370	Ala	Val	Lys	Gly	Phe 375	Tyr	Pro	Ser	Asp	Ile 380	Ala	Val	Glu	Trp	
Glu 385	Ser	Asn	Gly	Gln	Pro 390	Glu	Asn	Asn	Tyr	Lys 395	Thr	Thr	Pro	Pro	Val 400	
Leu	Asp	Ser	Asp	Gly 405	Ser	Phe	Phe	Leu	Val 410	Ser	Arg	Leu	Thr	Val 415	Asp	
Lys	Ser	Arg	Trp 420	Gln	Glu	Gly	Asn	Val 425	Phe	Ser	Сув	Ser	Val 430	Met	His	
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Tyr	Met	His 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Gln	Gly	Leu 45	Glu	Trp	Met	
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Ala	Arg	Ser	Pro 100	Asn	Pro	Tyr	Tyr	Tyr 105	Asp	Ser	Ser	Gly	Tyr 110	Tyr	Tyr
Pro	Gly	Ala 115	Phe	Asp	Ile	Trp	Gly 120	Gln	Gly	Thr	Met	Val 125	Thr	Val	Ser
Ser	Ala 130	Ser	Val	Ala	Ala	Pro 135	Ser	Val	Phe	Ile	Phe 140	Pro	Pro	Ser	Asp
Glu 145	Gln	Leu	Lys	Ser	Gly 150	Thr	Ala	Ser	Val	Val 155	Cys	Leu	Leu	Asn	Asn 160
Phe	Tyr	Pro	Arg	Glu 165	Ala	Lys	Val	Gln	Trp 170	Lys	Val	Asp	Asn	Ala 175	Leu
Gln	Ser	Gly	Asn 180	Ser	Gln	Glu	Ser	Val 185	Thr	Glu	Gln	Asp	Ser 190	Lys	Asp
Ser	Thr	Tyr 195	Ser	Leu	Ser	Ser	Thr 200	Leu	Thr	Leu	Ser	Lys 205	Ala	Asp	Tyr
Glu	Lys 210	His	Lys	Val	Tyr	Ala 215	Cys	Glu	Val	Thr	His 220	Gln	Gly	Leu	Ser
Ser 225	Pro	Val	Thr	Lys	Ser 230	Phe	Asn	Arg	Gly	Glu 235	CÀa	Pro	Pro	Cys	Pro 240
Pro	Cys	Pro	Ala	Pro 245	Glu	Phe	Glu	Gly	Gly 250	Pro	Ser	Val	Phe	Leu 255	Phe
Pro	Pro	Lys	Pro 260	Lys	Asp	Thr	Leu	Met 265	Ala	Ser	Arg	Thr	Pro 270	Glu	Val
Thr	Cys	Val 275	Val	Val	Asp	Val	Ser 280	Gln	Glu	Asp	Pro	Glu 285	Val	Gln	Phe
Asn	Trp 290	Tyr	Val	Asp	Gly	Val 295	Glu	Val	His	Asn	Ala 300	Lys	Thr	Lys	Pro
Arg 305	Glu	Glu	Gln	Phe	Asn 310	Ser	Thr	Tyr	Arg	Val 315	Val	Ser	Val	Leu	Thr 320
Val	Leu	Ala	Gln	Asp 325	Trp	Leu	Asn	Gly	330 Lys	Glu	Tyr	Lys	Cys	Lys 335	Val
Ser	Asn	Lys	Gly 340	Leu	Pro	Ser	Ser	Ile 345	Glu	Lys	Thr	Ile	Ser 350	Lys	Ala
Lys	Gly	Gln 355	Pro	Arg	Glu	Pro	Gln 360	Val	Tyr	Thr	Leu	Pro 365	Pro	Сув	Gln
Glu	Glu 370	Met	Thr	Lys	Asn	Gln 375	Val	Ser	Leu	Trp	Cys	Leu	Val	Lys	Gly
Phe 385	Tyr	Pro	Ser	Asp	Ile 390	Ala	Val	Glu	Trp	Glu 395	Ser	Asn	Gly	Gln	Pro 400
Glu	Asn	Asn	Tyr	Lys 405	Thr	Thr	Pro	Pro	Val 410	Leu	Asp	Ser	Asp	Gly 415	Ser
Phe	Phe	Leu	Tyr 420	Ser	Arg	Leu	Thr	Val 425	Asp	Lys	Ser	Arg	Trp 430	Gln	Glu
Gly	Asn	Val 435	Phe	Ser	Сув	Ser	Val 440	Met	His	Glu	Ala	Leu 445	His	Asn	Ala
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Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
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Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
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Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
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Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
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Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
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Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
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Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
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Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
                     55
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Trp	Val	Phe	Gly 100	Gly	Gly	Thr	Lys	Leu 105	Thr	Val	Leu	Ser	Ser 110	Ala	Ser
Thr	Lys	Gly 115	Pro	Ser	Val	Phe	Pro 120	Leu	Ala	Pro	Cys	Ser 125	Arg	Ser	Thr
Ser	Glu 130	Ser	Thr	Ala	Ala	Leu 135	Gly	Сув	Leu	Val	Lys 140	Asp	Tyr	Phe	Pro
Glu 145	Pro	Val	Thr	Val	Ser 150	Trp	Asn	Ser	Gly	Ala 155	Leu	Thr	Ser	Gly	Val 160
His	Thr	Phe	Pro	Ala 165	Val	Leu	Gln	Ser	Ser 170	Gly	Leu	Tyr	Ser	Leu 175	Ser
Ser	Val	Val	Thr 180	Val	Pro	Ser	Ser	Ser 185	Leu	Gly	Thr	Lys	Thr 190	Tyr	Thr
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Gly	Trp 50	Ile	Asn	Thr	Tyr	Thr 55	Gly	Glu	Pro	Thr	Tyr 60	Ala	Ala	Asp	Phe
Lys	Arg	Arg	Phe	Thr	Phe 70	Ser	Leu	Asp	Thr	Ser 75	Lys	Ser	Thr	Ala	Tyr 80
Leu	Gln	Met	Asn	Ser 85	Leu	Arg	Ala	Glu	90	Thr	Ala	Val	Tyr	Tyr 95	Càa
Ala	Lys	Tyr	Pro 100	Tyr	Tyr	Tyr	Gly	Thr 105	Ser	His	Trp	Tyr	Phe 110	Asp	Val
Trp	Gly	Gln 115	Gly	Thr	Leu	Val	Thr 120	Val	Ser	Ser	Ala	Ser 125	Thr	Lys	Gly
Pro	Ser 130	Val	Phe	Pro	Leu	Ala 135	Pro	Ser	Ser	Lys	Ser 140	Thr	Ser	Gly	Gly
Thr 145	Ala	Ala	Leu	Gly	Cys 150	Leu	Val	Lys	Asp	Tyr 155	Phe	Pro	Glu	Pro	Val 160
Thr	Val	Ser	Trp	Asn 165	Ser	Gly	Ala	Leu	Thr 170	Ser	Gly	Val	His	Thr 175	Phe
Pro		7707	T.011	C1 5	Cor	Ser	Glv	Lou	m	a	T	G	G		

200 Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu 230 235 Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ala Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser 290 295 Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu Ala Gln Asp Trp Leu 310 315 Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro 345 Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln 360 Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala 375 Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr 390 395 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu 405 410 Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser 425 Val Met His Glu Ala Leu His Asn Ala Tyr Thr Gln Lys Ser Leu Ser 440 Leu Ser Pro Gly Lys 450 <210> SEQ ID NO 47 <211> LENGTH: 705 <212> TYPE: PRT <213> ORGANISM: Artificial <223> OTHER INFORMATION: Heavy chain 2 of <VEGF-ANG-2> OAscFab IgG1 with AAA mutations <400> SEQUENCE: 47 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln 10 Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val 25 His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val

65					70					75					80
	Glu	Ala	Asp	Tyr 85	Tyr	Cys	Gln	Val	Trp 90	Asp	Ser	Ser	Ser	Asp 95	His
Trp	Val	Phe	Gly 100	Gly	Gly	Thr	Lys	Leu 105	Thr	Val	Leu	Gly	Gln 110	Pro	ГЛа
Ala	Ala	Pro 115	Ser	Val	Thr	Leu	Phe 120	Pro	Pro	Ser	Ser	Glu 125	Glu	Leu	Gln
Ala	Asn 130	Lys	Ala	Thr	Leu	Val 135	Cys	Leu	Ile	Ser	Asp 140	Phe	Tyr	Pro	Gly
Ala 145	Val	Thr	Val	Ala	Trp 150	Lys	Ala	Asp	Ser	Ser 155	Pro	Val	Lys	Ala	Gly 160
Val	Glu	Thr	Thr	Thr 165	Pro	Ser	Lys	Gln	Ser 170	Asn	Asn	ГÀа	Tyr	Ala 175	Ala
Ser	Ser	Tyr	Leu 180	Ser	Leu	Thr	Pro	Glu 185	Gln	Trp	Lys	Ser	His 190	Arg	Ser
Tyr	Ser	Сув 195	Gln	Val	Thr	His	Glu 200	Gly	Ser	Thr	Val	Glu 205	Lys	Thr	Val
Ala	Pro 210	Thr	Glu	CAa	Ser	Gly 215	Gly	Gly	Gly	Ser	Gly 220	Gly	Gly	Gly	Ser
Gly 225	Gly	Gly	Gly	Ser	Gly 230	Gly	Gly	Gly	Ser	Gly 235	Gly	Gly	Gly	Ser	Gly 240
Gly	Gly	Gly	Ser	Gly 245	Gly	Gln	Val	Gln	Leu 250	Val	Glu	Ser	Gly	Ala 255	Glu
Val	Lys	Lys	Pro 260	Gly	Ala	Ser	Val	Lys 265	Val	Ser	CÀa	ГÀв	Ala 270	Ser	Gly
Tyr	Thr	Phe 275	Thr	Gly	Tyr	Tyr	Met 280	His	Trp	Val	Arg	Gln 285	Ala	Pro	Gly
Gln	Gly 290	Leu	Glu	Trp	Met	Gly 295	Trp	Ile	Asn	Pro	Asn 300	Ser	Gly	Gly	Thr
Asn 305	Tyr	Ala	Gln	ГÀз	Phe 310	Gln	Gly	Arg	Val	Thr 315	Met	Thr	Arg	Asp	Thr 320
Ser	Ile	Ser	Thr	Ala 325	Tyr	Met	Glu	Leu	Ser 330	Arg	Leu	Arg	Ser	335	Asp
Thr	Ala	Val	Tyr 340	Tyr	CAa	Ala	Arg	Ser 345	Pro	Asn	Pro	Tyr	Tyr 350	Tyr	Asp
Ser	Ser	Gly 355	Tyr	Tyr	Tyr	Pro	Gly 360	Ala	Phe	Asp	Ile	Trp 365	Gly	Gln	Gly
Thr	Met 370	Val	Thr	Val	Ser	Ser 375	Ala	Ser	Thr	Lys	Gly 380	Pro	Ser	Val	Phe
Pro 385	Leu	Ala	Pro	Ser	Ser 390	Lys	Ser	Thr	Ser	Gly 395	Gly	Thr	Ala	Ala	Leu 400
Gly	Cha	Leu	Val	Lys 405	Asp	Tyr	Phe	Pro	Glu 410	Pro	Val	Thr	Val	Ser 415	Trp
Asn	Ser	Gly	Ala 420	Leu	Thr	Ser	Gly	Val 425	His	Thr	Phe	Pro	Ala 430	Val	Leu
Gln	Ser	Ser 435	Gly	Leu	Tyr	Ser	Leu 440	Ser	Ser	Val	Val	Thr 445	Val	Pro	Ser
Ser	Ser 450	Leu	Gly	Thr	Gln	Thr 455	Tyr	Ile	Сув	Asn	Val 460	Asn	His	Lys	Pro
Ser 465	Asn	Thr	Lys	Val	Asp 470	Lys	Lys	Val	Glu	Pro 475	Lys	Ser	Сув	Asp	Lys 480

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro 490 Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ala Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp 520 Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu Ala Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys 585 590 Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr 600 Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp 615 Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu 630 635 Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu 650 Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys 665 Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu 680 Ala Leu His Asn Ala Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly 695 Lys 705 <210> SEQ ID NO 48 <211> LENGTH: 214 <212> TYPE: PRT <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: Light chain 1 of <VEGF-ANG-2> OAscFab IgG1 with AAA mutations <400> SEQUENCE: 48 Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly 55 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala

			100					105					110		
Pro	Ser	Val 115	Phe	Ile	Phe	Pro	Pro 120	Ser	Asp	Glu	Gln	Leu 125	Lys	Ser	Gly
Thr	Ala 130	Ser	Val	Val	Cys	Leu 135	Leu	Asn	Asn	Phe	Tyr 140	Pro	Arg	Glu	Ala
Lys 145	Val	Gln	Trp	ràa	Val 150	Asp	Asn	Ala	Leu	Gln 155	Ser	Gly	Asn	Ser	Gln 160
Glu	Ser	Val	Thr	Glu 165	Gln	Asp	Ser	Lys	Asp 170	Ser	Thr	Tyr	Ser	Leu 175	Ser
Ser	Thr	Leu	Thr 180	Leu	Ser	ГÀа	Ala	Asp 185	Tyr	Glu	Lys	His	Lys 190	Val	Tyr
Ala	Cha	Glu 195	Val	Thr	His	Gln	Gly 200	Leu	Ser	Ser	Pro	Val 205	Thr	Lys	Ser
Phe	Asn 210	Arg	Gly	Glu	Cys										
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Ser	Leu	Arg	Leu 20	Ser	CAa	Ala	Ala	Ser 25	Gly	Tyr	Asp	Phe	Thr 30	His	Tyr
Gly	Met	Asn 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Val
Gly	Trp 50	Ile	Asn	Thr	Tyr	Thr 55	Gly	Glu	Pro	Thr	Tyr 60	Ala	Ala	Asp	Phe
Lys 65	Arg	Arg	Phe	Thr	Phe 70	Ser	Leu	Asp	Thr	Ser 75	Lys	Ser	Thr	Ala	Tyr 80
Leu	Gln	Met	Asn	Ser 85	Leu	Arg	Ala	Glu	Asp 90	Thr	Ala	Val	Tyr	Tyr 95	Cys
Ala	ГÀа	Tyr	Pro 100	Tyr	Tyr	Tyr	Gly	Thr 105	Ser	His	Trp	Tyr	Phe 110	Asp	Val
Trp	Gly	Gln 115	Gly	Thr	Leu	Val	Thr 120	Val	Ser	Ser	Ala	Ser 125	Thr	Lys	Gly
Pro	Ser 130	Val	Phe	Pro	Leu	Ala 135	Pro	Cys	Ser	Arg	Ser 140	Thr	Ser	Glu	Ser
Thr 145	Ala	Ala	Leu	Gly	Cys 150	Leu	Val	Lys	Asp	Tyr 155	Phe	Pro	Glu	Pro	Val 160
Thr	Val	Ser	Trp	Asn 165	Ser	Gly	Ala	Leu	Thr 170	Ser	Gly	Val	His	Thr 175	Phe
Pro	Ala	Val	Leu 180	Gln	Ser	Ser	Gly	Leu 185	Tyr	Ser	Leu	Ser	Ser 190	Val	Val
Thr	Val	Pro 195	Ser	Ser	Ser	Leu	Gly 200	Thr	Lys	Thr	Tyr	Thr 205	Сув	Asn	Val
Asp	His 210	Lys	Pro	Ser	Asn	Thr 215	Lys	Val	Asp	Lys	Arg 220	Val	Glu	Ser	Lys

Tyr Gly P 225	ro Pro	Cys	Pro 230	Pro	Cys	Pro	Ala	Pro 235	Glu	Phe	Glu	Gly	Gly 240
Pro Ser V	al Phe	Leu 245	Phe	Pro	Pro	Lys	Pro 250	Lys	Asp	Thr	Leu	Met 255	Ala
Ser Arg T	nr Pro 260		Val	Thr	Cys	Val 265		Val	Asp	Val	Ser 270	Gln	Glu
Asp Pro G	lu Val 75	Gln	Phe	Asn	Trp 280		Val	Asp	Gly	Val 285	Glu	Val	His
Asn Ala L	s Thr	Lys	Pro	Arg 295	Glu	Glu	Gln	Phe	Asn 300	Ser	Thr	Tyr	Arg
Val Val S 305	er Val	Leu	Thr 310	Val	Leu	Ala	Gln	Asp 315		Leu	Asn	Gly	Lys 320
Glu Tyr L	va CAa	Lys 325	Val	Ser	Asn	Lys	Gly 330	Leu	Pro	Ser	Ser	Ile 335	Glu
Lys Thr I	Le Ser 340		Ala	Lys	Gly	Gln 345	Pro	Arg	Glu	Pro	Gln 350	Val	Cha
Thr Leu P	ro Pro 55	Ser	Gln	Glu	Glu 360	Met	Thr	Lys	Asn	Gln 365	Val	Ser	Leu
Ser Cys A 370	la Val	Lys	Gly	Phe 375		Pro	Ser	Asp	Ile 380	Ala	Val	Glu	Trp
Glu Ser A 385	en Gly	Gln	Pro 390	Glu	Asn	Asn	Tyr	Lys 395	Thr	Thr	Pro	Pro	Val 400
Leu Asp S	er Asp	Gly 405	Ser	Phe	Phe	Leu	Val 410	Ser	Arg	Leu	Thr	Val 415	Asp
Lys Ser A	rg Trp 420		Glu	Gly	Asn	Val 425	Phe	Ser	CAa	Ser	Val 430	Met	His
Glu Ala L 4	eu His 35	Asn	Ala	Tyr	Thr 440	Gln	Lys	Ser	Leu	Ser 445	Leu	Ser	Leu
Gly Lys 450													
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His Trp T		Gln	Lys	Pro	Gly 40	Gln	Ala	Pro	Val	Leu 45	Val	Val	Tyr
Asp Asp S	er Asp	Arg	Pro	Ser 55	Gly	Ile	Pro	Glu	Arg 60	Phe	Ser	Gly	Ser
Asn Ser G	Ly Asn	Thr	Ala 70	Thr	Leu	Thr	Ile	Ser 75	Arg	Val	Glu	Ala	Gly 80
Asp Glu A	la Asp	Tyr 85	Tyr	Сув	Gln	Val	Trp 90	Asp	Ser	Ser	Ser	Asp 95	His
Trp Val P	ne Gly 100		Gly	Thr	Lys	Leu 105	Thr	Val	Leu	Gly	Gln 110	Pro	Lys

Ala	Ala	Pro 115	Ser	Val	Thr	Leu	Phe 120	Pro	Pro	Ser	Ser	Glu 125	Glu	Leu	Gln
Ala	Asn 130	Lys	Ala	Thr	Leu	Val 135	Cys	Leu	Ile	Ser	Asp 140	Phe	Tyr	Pro	Gly
Ala 145	Val	Thr	Val	Ala	Trp 150	Lys	Ala	Asp	Ser	Ser 155	Pro	Val	Lys	Ala	Gly 160
Val	Glu	Thr	Thr	Thr 165	Pro	Ser	Lys	Gln	Ser 170	Asn	Asn	ГÀа	Tyr	Ala 175	Ala
Ser	Ser	Tyr	Leu 180	Ser	Leu	Thr	Pro	Glu 185	Gln	Trp	Lys	Ser	His 190	Arg	Ser
Tyr	Ser	Суs 195	Gln	Val	Thr	His	Glu 200	Gly	Ser	Thr	Val	Glu 205	rys	Thr	Val
Ala	Pro 210	Thr	Glu	CÀa	Ser	Gly 215	Gly	Gly	Gly	Ser	Gly 220	Gly	Gly	Gly	Ser
Gly 225	Gly	Gly	Gly	Ser	Gly 230	Gly	Gly	Gly	Ser	Gly 235	Gly	Gly	Gly	Ser	Gly 240
Gly	Gly	Gly	Ser	Gly 245	Gly	Gln	Val	Gln	Leu 250	Val	Glu	Ser	Gly	Ala 255	Glu
Val	Lys	Lys	Pro 260	Gly	Ala	Ser	Val	Lys 265	Val	Ser	Cys	Lys	Ala 270	Ser	Gly
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Gln	Gly 290	Leu	Glu	Trp	Met	Gly 295	Trp	Ile	Asn	Pro	Asn 300	Ser	Gly	Gly	Thr
Asn 305	Tyr	Ala	Gln	Lys	Phe 310	Gln	Gly	Arg	Val	Thr 315	Met	Thr	Arg	Asp	Thr 320
Ser	Ile	Ser	Thr	Ala 325	Tyr	Met	Glu	Leu	Ser 330	Arg	Leu	Arg	Ser	Asp 335	Asp
Thr	Ala	Val	Tyr 340	Tyr	CÀa	Ala	Arg	Ser 345	Pro	Asn	Pro	Tyr	Tyr 350	Tyr	Asp
Ser	Ser	Gly 355	Tyr	Tyr	Tyr	Pro	Gly 360	Ala	Phe	Asp	Ile	Trp 365	Gly	Gln	Gly
Thr	Met 370	Val	Thr	Val	Ser	Ser 375	Ala	Ser	Thr	Lys	Gly 380	Pro	Ser	Val	Phe
Pro 385	Leu	Ala	Pro	СЛа	Ser 390	Arg	Ser	Thr	Ser	Glu 395	Ser	Thr	Ala	Ala	Leu 400
Gly	Cys	Leu	Val	Lys 405	Asp	Tyr	Phe	Pro	Glu 410	Pro	Val	Thr	Val	Ser 415	Trp
Asn	Ser	Gly	Ala 420	Leu	Thr	Ser	Gly	Val 425	His	Thr	Phe	Pro	Ala 430	Val	Leu
Gln	Ser	Ser 435	Gly	Leu	Tyr	Ser	Leu 440	Ser	Ser	Val	Val	Thr 445	Val	Pro	Ser
Ser	Ser 450	Leu	Gly	Thr	Lys	Thr 455	Tyr	Thr	Сув	Asn	Val 460	Asp	His	Lys	Pro
Ser 465	Asn	Thr	Lys	Val	Asp 470	Lys	Arg	Val	Glu	Ser 475	Lys	Tyr	Gly	Pro	Pro 480
Cys	Pro	Pro	Сув	Pro 485	Ala	Pro	Glu	Phe	Glu 490	Gly	Gly	Pro	Ser	Val 495	Phe
Leu	Phe	Pro	Pro 500	Lys	Pro	Lys	Asp	Thr 505	Leu	Met	Ala	Ser	Arg 510	Thr	Pro

	Val	Thr 515	CAa	Val	Val	Val	Asp 520	Val	Ser	Gln	Glu	Asp 525	Pro	Glu	Val
Gln	Phe 530	Asn	Trp	Tyr	Val	Asp 535	Gly	Val	Glu	Val	His 540	Asn	Ala	Lys	Thr
Lys 545	Pro	Arg	Glu	Glu	Gln 550	Phe	Asn	Ser	Thr	Tyr 555	Arg	Val	Val	Ser	Val 560
Leu	Thr	Val	Leu	Ala 565	Gln	Asp	Trp	Leu	Asn 570	Gly	Lys	Glu	Tyr	Lys 575	Сув
Lys	Val	Ser	Asn 580	ГÀз	Gly	Leu	Pro	Ser 585	Ser	Ile	Glu	ГÀз	Thr 590	Ile	Ser
Lys	Ala	Lys 595	Gly	Gln	Pro	Arg	Glu 600	Pro	Gln	Val	Tyr	Thr 605	Leu	Pro	Pro
Cys	Gln 610	Glu	Glu	Met	Thr	Lys 615	Asn	Gln	Val	Ser	Leu 620	Trp	CÀa	Leu	Val
Lys 625	Gly	Phe	Tyr	Pro	Ser 630	Asp	Ile	Ala	Val	Glu 635	Trp	Glu	Ser	Asn	Gly 640
Gln	Pro	Glu	Asn	Asn 645	Tyr	ГЛа	Thr	Thr	Pro 650	Pro	Val	Leu	Asp	Ser 655	Asp
Gly	Ser	Phe	Phe 660	Leu	Tyr	Ser	Arg	Leu 665	Thr	Val	Asp	ГÀа	Ser 670	Arg	Trp
Gln	Glu	Gly 675	Asn	Val	Phe	Ser	680 CAs	Ser	Val	Met	His	Glu 685	Ala	Leu	His
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Glu	Ser	Val	Thr	Glu 165	Gln	Asp	Ser	Lys	Asp 170	Ser	Thr	Tyr	Ser	Leu 175	Ser	
Ser	Thr	Leu	Thr 180	Leu	Ser	Lys	Ala	Asp 185	Tyr	Glu	Lys	His	Lys 190	Val	Tyr	
Ala	Сув	Glu 195	Val	Thr	His	Gln	Gly 200	Leu	Ser	Ser	Pro	Val 205	Thr	Lys	Ser	
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Gly	Met	Asn 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Val	
Gly	Trp 50	Ile	Asn	Thr	Tyr	Thr 55	Gly	Glu	Pro	Thr	Tyr 60	Ala	Ala	Asp	Phe	
Lys 65	Arg	Arg	Phe	Thr	Phe 70	Ser	Leu	Asp	Thr	Ser 75	Lys	Ser	Thr	Ala	Tyr 80	
Leu	Gln	Met	Asn	Ser 85	Leu	Arg	Ala	Glu	Asp 90	Thr	Ala	Val	Tyr	Tyr 95	Cys	
Ala	Lys	Tyr	Pro 100	Tyr	Tyr	Tyr	Gly	Thr 105	Ser	His	Trp	Tyr	Phe 110	Asp	Val	
Trp	Gly	Gln 115	Gly	Thr	Leu	Val	Thr 120	Val	Ser	Ser	Ala	Ser 125	Thr	Lys	Gly	
Pro	Ser 130	Val	Phe	Pro	Leu	Ala 135	Pro	Ser	Ser	Lys	Ser 140	Thr	Ser	Gly	Gly	
Thr 145	Ala	Ala	Leu	Gly	Суз 150	Leu	Val	Lys	Asp	Tyr 155	Phe	Pro	Glu	Pro	Val 160	
Thr	Val	Ser	Trp	Asn 165	Ser	Gly	Ala	Leu	Thr 170	Ser	Gly	Val	His	Thr 175	Phe	
Pro	Ala	Val	Leu 180	Gln	Ser	Ser	Gly	Leu 185	Tyr	Ser	Leu	Ser	Ser 190	Val	Val	
Thr	Val	Pro 195	Ser	Ser	Ser	Leu	Gly 200	Thr	Gln	Thr	Tyr	Ile 205	Сув	Asn	Val	
Asn	His 210	Lys	Pro	Ser	Asn	Thr 215	Lys	Val	Asp	Lys	Lys 220	Val	Glu	Pro	Lys	
Ser 225	Cha	Asp	Lys	Thr	His 230	Thr	Cys	Pro	Pro	Cys 235	Pro	Ala	Pro	Glu	Leu 240	
Leu	Gly	Gly	Pro	Ser 245	Val	Phe	Leu	Phe	Pro 250	Pro	ГÀа	Pro	TÀa	Asp 255	Thr	
Leu	Met	Ile	Ser 260	Arg	Thr	Pro	Glu	Val 265	Thr	Cha	Val	Val	Val 270	Asp	Val	
Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	

		275					280					285			
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Thr 305	Tyr	Arg	Val	Val	Ser 310	Val	Leu	Thr	Val	Leu 315	His	Gln	Asp	Trp	Leu 320
Asn	Gly	Lys	Glu	Tyr 325	Lys	CAa	Lys	Val	Ser 330	Asn	ГÀа	Ala	Leu	Pro 335	Ala
Pro	Ile	Glu	Lys 340	Thr	Ile	Ser	Lys	Ala 345	Lys	Gly	Gln	Pro	Arg 350	Glu	Pro
Gln	Val	Tyr 355	Thr	Leu	Pro	Pro	Cys	Arg	Asp	Glu	Leu	Thr 365	Lys	Asn	Gln
Val	Ser 370	Leu	Trp	Cya	Leu	Val 375	_	Gly	Phe	Tyr	Pro 380	Ser	Asp	Ile	Ala
Val 385	Glu	Trp	Glu	Ser	Asn 390	Gly	Gln	Pro	Glu	Asn 395	Asn	Tyr	Lys	Thr	Thr 400
	Pro	Val	Leu	Asp		Asp	Gly	Ser	Phe		Leu	Tyr	Ser	Lys 415	
Thr	Val	Asp	Lys 420		Arg	Trp	Gln	Gln 425		Asn	Val	Phe	Ser 430		Ser
Val	Met	His		Ala	Leu	His			Tyr	Thr	Gln	_		Leu	Ser
Leu	Ser 450		Gly	ГÀв			440					445			
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Ser	Val	Lys	Val 20	Ser	Cys	Lys	Ala	Ser 25	Gly	Tyr	Thr	Phe	Thr	Gly	Tyr
Tyr	Met	His 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Gln	Gly	Leu 45	Glu	Trp	Met
_	Trp 50	Ile	Asn	Pro	Asn	Ser 55	Gly	Gly	Thr	Asn	Tyr 60	Ala	Gln	Lys	Phe
Gln 65	Gly	Arg	Val	Thr	Met 70	Thr	Arg	Asp	Thr	Ser 75	Ile	Ser	Thr	Ala	Tyr 80
Met	Glu	Leu	Ser	Arg 85	Leu	Arg	Ser	Asp	Asp	Thr	Ala	Val	Tyr	Tyr 95	Cys
Ala	Arg	Ser	Pro	Asn	Pro	Tyr	Tyr	Tyr 105	Asp	Ser	Ser	Gly	Tyr 110	Tyr	Tyr
Pro	Gly	Ala 115	Phe	Asp	Ile	Trp	Gly 120	Gln	Gly	Thr	Met	Val 125	Thr	Val	Ser
	Ala 130	Ser	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp
		Leu	Lys	Ser	Gly 150	Thr	Ala	Ser	Val	Val 155		Leu	Leu	Asn	Asn 160
-13															

Phe	Tyr	Pro	Arg	Glu 165	Ala	Lys	Val	Gln	Trp 170	Lys	Val	Asp	Asn	Ala 175	Leu	
Gln	Ser	Gly	Asn 180	Ser	Gln	Glu	Ser	Val 185	Thr	Glu	Gln	Asp	Ser 190	ГЛа	Asp	
Ser	Thr	Tyr 195	Ser	Leu	Ser	Ser	Thr 200	Leu	Thr	Leu	Ser	Lys 205	Ala	Asp	Tyr	
Glu	Lys 210	His	Lys	Val	Tyr	Ala 215	Cys	Glu	Val	Thr	His 220	Gln	Gly	Leu	Ser	
Ser 225	Pro	Val	Thr	Lys	Ser 230	Phe	Asn	Arg	Gly	Glu 235	Сув	Asp	Lys	Thr	His 240	
Thr	Cys	Pro	Pro	Cys 245	Pro	Ala	Pro	Glu	Leu 250	Leu	Gly	Gly	Pro	Ser 255	Val	
Phe	Leu	Phe	Pro 260	Pro	Lys	Pro	Lys	Asp 265	Thr	Leu	Met	Ile	Ser 270	Arg	Thr	
Pro	Glu	Val 275	Thr	Cys	Val	Val	Val 280	Asp	Val	Ser	His	Glu 285	Asp	Pro	Glu	
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Thr 305	Lys	Pro	Arg	Glu	Glu 310	Gln	Tyr	Asn	Ser	Thr 315	Tyr	Arg	Val	Val	Ser 320	
Val	Leu	Thr	Val	Leu 325	His	Gln	Asp	Trp	Leu 330	Asn	Gly	Lys	Glu	Tyr 335	Lys	
CÀa	Lys	Val	Ser 340	Asn	Lys	Ala	Leu	Pro 345	Ala	Pro	Ile	Glu	Lys 350	Thr	Ile	
Ser	Lys	Ala 355		Gly	Gln	Pro	Arg 360	Glu	Pro	Gln	Val	Сув 365	Thr	Leu	Pro	
Pro	Ser 370	Arg	Asp	Glu	Leu	Thr 375	Lys	Asn	Gln	Val	Ser 380	Leu	Ser	СЛв	Ala	
Val 385	Lys	Gly	Phe	Tyr	Pro 390	Ser	Asp	Ile	Ala	Val 395	Glu	Trp	Glu	Ser	Asn 400	
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Asp	Gly	Ser	Phe 420	Phe	Leu	Val	Ser	Lys 425	Leu	Thr	Val	Asp	Lys 430	Ser	Arg	
Trp	Gln	Gln 435	Gly	Asn	Val	Phe	Ser 440	Cys	Ser	Val	Met	His 445	Glu	Ala	Leu	
His		His	-		Gln	•					Ser 460		Gly	Lys		
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Asp 1	Ile	Gln	Leu	Thr 5	Gln	Ser	Pro	Ser	Ser 10	Leu	Ser	Ala	Ser	Val 15	Gly	
Asp	Arg	Val	Thr 20	Ile	Thr	Cys	Ser	Ala 25	Ser	Gln	Asp	Ile	Ser 30	Asn	Tyr	
Leu	Asn	Trp 35	Tyr	Gln	Gln	Lys	Pro 40	Gly	Lys	Ala	Pro	Lуs 45	Val	Leu	Ile	

ıyı	50	1111	Set	Ser	пец	55	Set	GIY	vai	FIO	60	AIG	FIIC	Set	GIY	
Ser 65	Gly	Ser	Gly	Thr	Asp 70	Phe	Thr	Leu	Thr	Ile 75	Ser	Ser	Leu	Gln	Pro 80	
Glu	Asp	Phe	Ala	Thr 85	Tyr	Tyr	Сув	Gln	Gln 90	Tyr	Ser	Thr	Val	Pro 95	Trp	
Thr	Phe	Gly	Gln 100	Gly	Thr	Lys	Val	Glu 105	Ile	rys	Arg	Thr	Val 110	Ala	Ala	
Pro	Ser	Val 115	Phe	Ile	Phe	Pro	Pro 120	Ser	Asp	Glu	Gln	Leu 125	Lys	Ser	Gly	
Thr	Ala 130	Ser	Val	Val	Сув	Leu 135	Leu	Asn	Asn	Phe	Tyr 140	Pro	Arg	Glu	Ala	
Lys 145	Val	Gln	Trp	Lys	Val 150	Asp	Asn	Ala	Leu	Gln 155	Ser	Gly	Asn	Ser	Gln 160	
Glu	Ser	Val	Thr	Glu 165	Gln	Asp	Ser	Lys	Asp 170	Ser	Thr	Tyr	Ser	Leu 175	Ser	
Ser	Thr	Leu	Thr 180	Leu	Ser	Lys	Ala	Asp 185	Tyr	Glu	Lys	His	Lys 190	Val	Tyr	
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Phe	Asn 210	Arg	Gly	Glu	Cys											
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Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly

His	Thr	Phe	Pro	Ala 165	Val	Leu	Gln	Ser	Ser 170	Gly	Leu	Tyr	Ser	Leu 175	Ser	
Ser	Val	Val	Thr 180	Val	Pro	Ser	Ser	Ser 185	Leu	Gly	Thr	Gln	Thr 190	Tyr	Ile	
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	0> SI				a 1	a	G1	G 1	a 1	T	**- 7	a 1	D	G1	G1	
1	Val	GIN	ьeu	vai 5	GIU	ser	GIY	GIY	10	ьeu	vai	GIN	Pro	15	GIY	
Ser	Leu	Arg	Leu 20	Ser	CAa	Ala	Ala	Ser 25	Gly	Tyr	Asp	Phe	Thr 30	His	Tyr	
Gly	Met	Asn 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Val	
Gly	Trp 50	Ile	Asn	Thr	Tyr	Thr 55	Gly	Glu	Pro	Thr	Tyr 60	Ala	Ala	Asp	Phe	
Lys 65	Arg	Arg	Phe	Thr	Phe 70	Ser	Leu	Asp	Thr	Ser 75	ГÀа	Ser	Thr	Ala	Tyr 80	
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Ala	ГЛа	Tyr	Pro 100	Tyr	Tyr	Tyr	Gly	Thr 105	Ser	His	Trp	Tyr	Phe 110	Asp	Val	
Trp	Gly	Gln 115	Gly	Thr	Leu	Val	Thr 120	Val	Ser	Ser	Ala	Ser 125	Thr	Lys	Gly	
Pro	Ser 130	Val	Phe	Pro	Leu	Ala 135	Pro	Ser	Ser	Lys	Ser 140	Thr	Ser	Gly	Gly	
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Thr	Val	Ser	Trp	Asn 165	Ser	Gly	Ala	Leu	Thr 170	Ser	Gly	Val	His	Thr 175	Phe	
Pro	Ala	Val	Leu 180	Gln	Ser	Ser	Gly	Leu 185	Tyr	Ser	Leu	Ser	Ser 190	Val	Val	
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Ser 225	Сув	Asp	Lys	Thr	His 230	Thr	Cys	Pro	Pro	Сув 235	Pro	Ala	Pro	Glu	Ala 240	
Ala	Gly	Gly	Pro	Ser 245	Val	Phe	Leu	Phe	Pro 250	Pro	ГÀв	Pro	Lys	Asp 255	Thr	
Leu	Met	Ile	Ser 260	Arg	Thr	Pro	Glu	Val 265	Thr	Сув	Val	Val	Val 270	Asp	Val	
Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	

### Case																
## 290			275					280					285			
310 315	Glu		His	Asn	Ala	rys		Lys	Pro	Arg	Glu		Gln	Tyr	Asn	Ser
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Arg Sas		_	Arg	Val	Val		Val	Leu	Thr	Val		His	Gln	Asp	Trp	
Sado	Asn	Gly	Lys	Glu	_	Lys	Cys	Lys	Val		Asn	Lys	Ala	Leu	_	Ala
Nai	Pro	Ile	Glu	_	Thr	Ile	Ser	ГЛа		Lys	Gly	Gln	Pro	_	Glu	Pro
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr 385	Gln	Val		Thr	Leu	Pro	Pro		Arg	Asp	Glu	Leu		Lys	Asn	Gln
390 395 400 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Alas Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Alas Mathematical Cycles Features:	Val		Leu	Trp	CÀa	Leu		Lys	Gly	Phe	Tyr		Ser	Asp	Ile	Ala
## 105 ## 1410 ## 1415 ## 1520 ## 1415 ## 1520			Trp	Glu	Ser		Gly	Gln	Pro	Glu		Asn	Tyr	Lys	Thr	
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser 435	Pro	Pro	Val	Leu	_	Ser	Asp	Gly	Ser		Phe	Leu	Tyr	Ser	-	Leu
Leu Ser Pro Gly Lys 450	Thr	Val	Asp	-	Ser	Arg	Trp	Gln		Gly	Asn	Val	Phe		Cys	Ser
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Gln Val Gln Leu Val Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Gly Ala Glu Val Lys Lys Pro Gly Ala Gly Ala Ser Gly Tyr Thr Phe Thr Gly Tyr Gly Tyr Mar Ala Gly Tyr Mar Thr Mar Gly Tyr Mar Ala Gly Gly Gln Gly Gly Glu Gly	<223	ro < 6 iw 7)	THER Lth I VEGF	INFO 23290 ang2-	G LAI -0015	LA mi										
10																
20	1					Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ala
So So So So So So So So			-, -	Val				-		10		-	-		15	
So	1,377	Met	Hie	20	Ser	Сув	Lys	Ala	Ser 25	10 Gly	Tyr	Thr	Phe	Thr 30	15 Gly	Tyr
65 70 75 80 Met Glu Leu Ser Arg Leu Arg Ser Asp go Thr Ala Val Tyr Tyr Cys g5 25 Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr 100 Pro Gly Ala Phe Asp Ile Trp Gly Gly Gln Gly Thr Met Val Tyr Val Ser Ser Ala Ser Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp			35	20 Trp	Ser Val	Cys Arg	Lys Gln	Ala Ala 40	Ser 25 Pro	10 Gly Gly	Tyr Gln	Thr Gly	Phe Leu 45	Thr 30 Glu	Gly Trp	Tyr Met
85 90 95 Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr 110 Fro Gly Ala Phe Asp Ile Trp Gly Gly Gln Gly Thr Met Val Thr Val Ser 125 Ser Ala Ser Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp	Gly	Trp 50	35 Ile	20 Trp Asn	Ser Val Pro	Cys Arg Asn	Lys Gln Ser 55	Ala Ala 40 Gly	Ser 25 Pro Gly	10 Gly Gly Thr	Tyr Gln Asn	Thr Gly Tyr 60	Phe Leu 45 Ala	Thr 30 Glu Gln	Gly Trp Lys	Tyr Met Phe
Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser 115 Ser Ala Ser Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp	Gly Gln 65	Trp 50 Gly	35 Ile Arg	20 Trp Asn Val	Ser Val Pro Thr	Cys Arg Asn Met 70	Lys Gln Ser 55 Thr	Ala Ala 40 Gly	Ser 25 Pro Gly Asp	Gly Gly Thr	Tyr Gln Asn Ser 75	Thr Gly Tyr 60	Phe Leu 45 Ala Ser	Thr 30 Glu Gln Thr	Gly Trp Lys Ala	Tyr Met Phe Tyr 80
115 120 125 Ser Ala Ser Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp	Gly Gln 65	Trp 50 Gly	35 Ile Arg	20 Trp Asn Val	Ser Val Pro Thr	Cys Arg Asn Met 70	Lys Gln Ser 55 Thr	Ala Ala 40 Gly	Ser 25 Pro Gly Asp	Gly Gly Thr Asp	Tyr Gln Asn Ser 75	Thr Gly Tyr 60	Phe Leu 45 Ala Ser	Thr 30 Glu Gln Thr	Gly Trp Lys Ala	Tyr Met Phe Tyr 80
-	Gly Gln 65 Met	Trp 50 Gly Glu	35 Ile Arg Leu	20 Trp Asn Val Ser	Ser Val Pro Thr Arg 85	Cys Arg Asn Met 70 Leu	Lys Gln Ser 55 Thr	Ala Ala 40 Gly Arg	Ser 25 Pro Gly Asp Asp	Gly Gly Thr Thr	Tyr Gln Asn Ser 75	Thr Gly Tyr 60 Ile	Phe Leu 45 Ala Ser	Thr 30 Glu Gln Thr Tyr	Gly Trp Lys Ala Tyr 95	Tyr Met Phe Tyr 80 Cys
	Gly Gln 65 Met	Trp 50 Gly Glu Arg	35 Ile Arg Leu Ser	20 Trp Asn Val Ser Pro	Ser Val Pro Thr Arg 85 Asn	Cys Arg Asn Met 70 Leu Pro	Lys Gln Ser 55 Thr Arg	Ala Ala 40 Gly Arg Ser Tyr	Ser 25 Pro Gly Asp Asp	Gly Gly Thr Asp 90 Asp	Tyr Gln Asn Ser 75 Thr	Thr Gly Tyr 60 Ile Ala	Phe Leu 45 Ala Ser Val Gly	Thr 30 Glu Gln Thr Tyr Tyr 110	Gly Trp Lys Ala Tyr 95 Tyr	Tyr Met Phe Tyr 80 Cys
Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn 145 $$ 150 $$ 155 $$ 160	Gly Gln 65 Met Ala	Trp 50 Gly Glu Arg Gly	35 Ile Arg Leu Ser Ala 115	20 Trp Asn Val Ser Pro 100	Ser Val Pro Thr Arg 85 Asn	Cys Arg Asn Met 70 Leu Pro	Lys Gln Ser 55 Thr Arg Tyr Trp	Ala Ala 40 Gly Arg Ser Tyr	Ser 25 Pro Gly Asp Tyr 105 Gln	10 Gly Gly Thr Thr Asp 90 Asp	Tyr Gln Asn Ser 75 Thr Ser	Thr Gly Tyr 60 Ile Ala Ser Met	Phe Leu 45 Ala Ser Val Gly Val 125	Thr 30 Glu Gln Thr Tyr Tyr 110 Thr	15 Gly Trp Lys Ala Tyr 95 Tyr	Tyr Met Phe Tyr 80 Cys Tyr

Phe	Tyr	Pro	Arg	Glu 165	Ala	Lys	Val	Gln	Trp 170	Lys	Val	Asp	Asn	Ala 175	Leu	
Gln	Ser	Gly	Asn 180	Ser	Gln	Glu	Ser	Val 185		Glu	Gln	Asp	Ser 190		Asp	
Ser	Thr	Tyr 195	Ser	Leu	Ser	Ser	Thr 200	Leu	Thr	Leu	Ser	Lys 205	Ala	Asp	Tyr	
Glu	Lys 210	His	Lys	Val	Tyr	Ala 215	СЛа	Glu	Val	Thr	His 220	Gln	Gly	Leu	Ser	
Ser 225	Pro	Val	Thr	Lys	Ser 230	Phe	Asn	Arg	Gly	Glu 235	СЛа	Asp	Lys	Thr	His 240	
Thr	Сув	Pro	Pro	Cys 245	Pro	Ala	Pro	Glu	Ala 250	Ala	Gly	Gly	Pro	Ser 255	Val	
Phe	Leu	Phe	Pro 260	Pro	Lys	Pro	ГЛа	Asp 265	Thr	Leu	Met	Ile	Ser 270	Arg	Thr	
Pro	Glu	Val 275	Thr	Cys	Val	Val	Val 280	Asp	Val	Ser	His	Glu 285	Asp	Pro	Glu	
Val	Lys 290	Phe	Asn	Trp	Tyr	Val 295	Asp	Gly	Val	Glu	Val 300	His	Asn	Ala	Lys	
Thr 305	ГЛа	Pro	Arg	Glu	Glu 310	Gln	Tyr	Asn	Ser	Thr 315	Tyr	Arg	Val	Val	Ser 320	
Val	Leu	Thr	Val	Leu 325	His	Gln	Asp	Trp	Leu 330	Asn	Gly	ГÀв	Glu	Tyr 335	ГÀа	
CAa	Lys	Val	Ser 340	Asn	Lys	Ala	Leu	Gly 345	Ala	Pro	Ile	Glu	Lув 350	Thr	Ile	
Ser	Lys	Ala 355	Lys	Gly	Gln	Pro	Arg 360	Glu	Pro	Gln	Val	Сув 365	Thr	Leu	Pro	
Pro	Ser 370	Arg	Asp	Glu	Leu	Thr 375	Lys	Asn	Gln	Val	Ser 380	Leu	Ser	Cys	Ala	
Val 385	Lys	Gly	Phe	Tyr	Pro 390	Ser	Asp	Ile	Ala	Val 395	Glu	Trp	Glu	Ser	Asn 400	
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1	Ile	GIII	БСи	5	0111				10					15		

пец	ASII	35	IYI	GIII	GIII	пуъ	40	Gly	цуъ	AIA	FIO	45	vai	пец	116	
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Glu	Asp	Phe	Ala	Thr 85	Tyr	Tyr	Cys	Gln	Gln 90	Tyr	Ser	Thr	Val	Pro 95	Trp	
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Trp	Glu	Ser	Asn	Gly 165	Gln	Pro	Glu	Asn	Asn 170	Tyr	Lys	Thr	Thr	Pro 175	Pro
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ı Gly			20					25	-		Lys Val	_	30		
I Gly Ile	Ser	Arg 35	20 Thr	Pro	Glu	Val	Thr 40	25 Cys	Val	Val	-	Asp 45	30 Val	Ser	His
Gly Tle Glu	Ser Asp 50	Arg 35 Pro	20 Thr Glu	Pro Val	Glu Lys	Val Phe 55	Thr 40 Asn	25 Cys Trp	Val Tyr	Val Val	Val Asp	Asp 45 Gly	30 Val Val	Ser Glu	His Val
Gly Tle Glu His	Ser Asp 50 Asn	Arg 35 Pro Ala	20 Thr Glu Lys	Pro Val Thr	Glu Lys Lys 70	Val Phe 55 Pro	Thr 40 Asn Arg	25 Cys Trp Glu	Val Tyr Glu	Val Val Gln 75	Val Asp 60	Asp 45 Gly Asn	30 Val Val Ser	Ser Glu Thr	His Val Tyr 80
ile Elu His 55	Ser Asp 50 Asn Val	Arg 35 Pro Ala Val	20 Thr Glu Lys Ser	Pro Val Thr Val 85	Glu Lys Lys 70 Leu	Val Phe 55 Pro Thr	Thr 40 Asn Arg Val	25 Cys Trp Glu Leu	Val Tyr Glu His	Val Val Gln 75 Gln	Val Asp 60 Tyr	Asp 45 Gly Asn Trp	30 Val Val Ser Leu	Ser Glu Thr Asn 95	His Val Tyr 80 Gly
ile Elu His 55 Arg	Ser Asp 50 Asn Val	Arg 35 Pro Ala Val	Thr Glu Lys Ser Lys 100	Pro Val Thr Val 85 Cys	Glu Lys Lys 70 Leu Lys	Val Phe 55 Pro Thr	Thr 40 Asn Arg Val	25 Cys Trp Glu Leu Asn 105	Val Tyr Glu His 90 Lys	Val Val Gln 75 Gln Ala	Val Asp 60 Tyr Asp	Asp 45 Gly Asn Trp	30 Val Val Ser Leu Ala 110	Ser Glu Thr Asn 95	His Val Tyr 80 Gly Ile
Ile Ile Ile Arg	Ser Asp 50 Asn Val Glu Lys	Arg 35 Pro Ala Val Tyr Thr	20 Thr Glu Lys Ser Lys 100 Ile	Pro Val Thr Val 85 Cys	Glu Lys Lys 70 Leu Lys	Val Phe 55 Pro Thr Val	Thr 40 Asn Arg Val Ser Lys 120	25 Cys Trp Glu Leu Asn 105 Gly	Val Tyr Glu His 90 Lys	Val Val Gln 75 Gln Ala	Val Asp 60 Tyr Asp	Asp 45 Gly Asn Trp Pro	30 Val Val Ser Leu Ala 110	Ser Glu Thr Asn 95 Pro	His Val Tyr 80 Gly Ile Val
I Gly Ille Glu His Karg Lys Cys Leu	Asp 50 Asn Val Glu Lys Thr	Arg 35 Pro Ala Val Tyr Thr 115 Leu	20 Thr Glu Lys Ser Lys 100 Ile	Pro Val Thr Val 85 Cys Ser	Glu Lys 70 Leu Lys Ser	Val Phe 55 Pro Thr Val Ala Arg 135	Thr 40 Asn Arg Val Ser Lys 120 Asp	Cys Trp Glu Leu Asn 105 Gly Glu	Val Tyr Glu His 90 Lys Gln Leu	Val Val Gln 75 Gln Ala Pro	Val Asp 60 Tyr Asp Leu Arg	Asp 45 Gly Asn Trp Pro Glu 125 Asn	30 Val Val Ser Leu Ala 110 Pro	Ser Glu Thr Asn 95 Pro Gln Val	His Val Tyr 80 Gly Ile Val Ser
Ile Glu His 65 Arg Lys Cys Leu 145	Ser Asp 50 Asn Val Glu Lys Thr 130 Ser	Arg 35 Pro Ala Val Tyr Thr 115 Leu Cys	20 Thr Glu Lys Ser Lys 100 Ile Pro	Pro Val Thr Val 85 Cys Ser Pro Val	Glu Lys Lys 70 Leu Lys Lys Lys Ser Lys 150	Val Phe 55 Pro Thr Val Ala Arg 135 Gly	Thr 40 Asn Arg Val Ser Lys 120 Asp	25 Cys Trp Glu Leu Asn 105 Gly Glu	Val Tyr Glu His 90 Lys Gln Leu Pro	Val Val Gln 75 Gln Ala Pro Thr	Val Asp 60 Tyr Asp Leu Arg	Asp 45 Gly Asn Trp Pro Glu 125 Asn	30 Val Val Ser Leu Ala 110 Pro Gln Ala	Ser Glu Thr Asn 95 Pro Gln Val	His Val Tyr 80 Gly Ile Val Ser Glu 160
Ile	Ser Asp 50 Asn Val Glu Lys Thr 130 Ser Glu	Arg 35 Pro Ala Val Tyr Thr 115 Leu Cys	20 Thr Glu Lys Ser Lys 100 Ile Pro Ala Asn	Pro Val Thr Val 85 Cys Ser Pro Val Gly 165	Glu Lys Lys 70 Leu Lys Lys Lys Gln Gln	Val Phe 55 Pro Thr Val Ala Arg 135 Gly Pro	Thr 40 Asn Arg Val Ser Lys 120 Asp Phe Glu	25 Cys Trp Glu Leu Asn 105 Gly Glu Tyr	Val Tyr Glu His 90 Lys Gln Leu Pro Asn 170	Val Gln 75 Gln Ala Pro Thr Ser 155	Val Asp 60 Tyr Asp Leu Arg Lys 140 Asp	Asp 45 Gly Asn Trp Pro Glu 125 Asn Ile	30 Val Val Ser Leu Ala 110 Pro Gln Ala Thr	Ser Glu Thr Asn 95 Pro Gln Val Val Pro 175	His Val Tyr 80 Gly Ile Val Ser Glu 160 Pro
Ile Ile Ile Ile Isi	Ser Asp 50 Asn Val Glu Lys Thr 130 Ser Glu Leu	Arg 35 Pro Ala Val Tyr Thr 115 Leu Cys Ser	20 Thr Glu Lys Ser Lys 100 Ile Pro Ala Asn Ser 180	Pro Val Thr Val 85 Cys Ser Pro Val Gly 165 Asp	Glu Lys 70 Leu Lys Lys Ser Lys 150 Gln Gly	Val Phe 55 Pro Thr Val Ala Arg 135 Gly Pro	Thr 40 Asn Arg Val Ser Lys 120 Asp Phe Glu	25 Cys Trp Glu Leu Asn 105 Gly Glu Tyr Asn Phe 185	Val Tyr Glu His 90 Lys Gln Leu Pro Asn 170 Leu	Val Gln 75 Gln Ala Pro Thr Ser 155 Tyr	Val Asp 60 Tyr Asp Leu Arg Lys 140 Asp	Asp 45 Gly Asn Trp Pro Glu 125 Asn Ile Thr	30 Val Val Ser Leu Ala 110 Pro Gln Ala Thr Leu 190	Ser Glu Thr Asn 95 Pro Gln Val Val Pro 175 Thr	His Val Tyr 80 Gly Ile Val Ser Glu 160 Pro

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Pro Gly Lys
225
<210> SEQ ID NO 66
<211> LENGTH: 227
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human IgG1 Fc-region derived Fc-region
     polypeptide with S354C, T366W mutations
<400> SEQUENCE: 66
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Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
                     105
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
                       120
Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
                     135
Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
            165
                        170
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
                   185
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
Pro Gly Lys
225
<210> SEQ ID NO 67
<211> LENGTH: 227
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: human IgG1 Fc-region derived Fc-region
     polypeptide with L234A, L235A mutations and Y349C, T366S, L368A,
     Y407V mutations
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Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
         20
                   25
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Ile	Ser	Arg 35	Thr	Pro	Glu	Val	Thr 40	Сув	Val	Val	Val	Asp 45	Val	Ser	His
Glu	Asp 50	Pro	Glu	Val	Lys	Phe 55	Asn	Trp	Tyr	Val	Asp 60	Gly	Val	Glu	Val
His 65	Asn	Ala	Lys	Thr	Lys 70	Pro	Arg	Glu	Glu	Gln 75	Tyr	Asn	Ser	Thr	Tyr 80
Arg	Val	Val	Ser	Val 85	Leu	Thr	Val	Leu	His 90	Gln	Asp	Trp	Leu	Asn 95	Gly
ГЛа	Glu	Tyr	Lys 100	CAa	Lys	Val	Ser	Asn 105	ГЛа	Ala	Leu	Pro	Ala 110	Pro	Ile
Glu	Lys	Thr 115	Ile	Ser	Lys	Ala	Lys 120	Gly	Gln	Pro	Arg	Glu 125	Pro	Gln	Val
Cys	Thr 130	Leu	Pro	Pro	Ser	Arg 135	Asp	Glu	Leu	Thr	Lys 140	Asn	Gln	Val	Ser
Leu 145	Ser	CAa	Ala	Val	Lys 150	Gly	Phe	Tyr	Pro	Ser 155	Asp	Ile	Ala	Val	Glu 160
Trp	Glu	Ser	Asn	Gly 165	Gln	Pro	Glu	Asn	Asn 170	Tyr	Lys	Thr	Thr	Pro 175	Pro
Val	Leu	Asp	Ser 180	Aap	Gly	Ser	Phe	Phe 185	Leu	Val	Ser	Lys	Leu 190	Thr	Val
Asp	ГÀа	Ser 195	Arg	Trp	Gln	Gln	Gly 200	Asn	Val	Phe	Ser	Сув 205	Ser	Val	Met
His	Glu 210	Ala	Leu	His	Asn	His 215	Tyr	Thr	Gln	Lys	Ser 220	Leu	Ser	Leu	Ser
Pro 225	Gly	Lys													
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<211 <212 <213 <220	.> LE !> TY !> OF !> FE !> OT	NGTH PE: GANI ATUR HER	H: 22 PRT SM: RE: INFO	27 Arti DRMAT	ION:	hum	an I	gG1							gion cions
<211 <212 <213 <220 <223	.> LE !> TY !> OF !> FE !> OT	NGTH PE: GANI ATUR HER Olype	H: 22 PRT SM: E: INFO	27 Arti DRMAT de wi	ION:	hum	an I	gG1							
<211 <212 <213 <220 <223	-> LE -> TY -> OF -> OF 	ENGTH PE: CGANI EATUF CHER DIYPE	H: 22 PRT SM: EE: INFO Pptic	27 Arti DRMAT de wi	ION:	hum L23	nan I 34A,	gG1 L235	iA ar	nd S3	354C,	T36	56W n	nutat	ions
<211 <212 <213 <220 <223 <400 Asp	.> LE -> TY -> OF -> OT 	ENGTH PE: GANI EATUF HER DIYPE QUEN	PRT SM: SM: INFO Ptic	Arti Arti DRMAT de wi 68 Thr	TION: ith a	hum L23	nan 1 34A, Pro	gG1 L235 Cys	Pro	nd S3	854C, Pro	T36	6W m	Ala 15	ions
<211 <212 <213 <220 <223 <400 Asp 1 Gly	.> LE !> TY !> OF !> OT !> OT !> SE Lys	ENGTH TPE: CGANI CATUF THER CLYPE CQUEN Thr	H: 22 PRT PRT SM: E: INFO PTIO	Arti DRMAT de wi 68 Thr 5	TION: ith a	hum L23 Pro	nan I 34A, Pro	gg1 L235 Cys Pro 25	Pro 10 Lys	Ala Pro	Pro Lys	T36 Glu Asp	Ala Thr	Ala 15 Leu	Gly Met
<211 <212 <213 <220 <223 <400 Asp 1 Gly	.> LE -> TY -> OF -> OT 	ENGTHER SATURE S	H: 22 PRT SM: E: INFO Ptic His Val 20 Thr	Arti DRMAT de wi 68 Thr 5 Phe	TION: ith a Cys Leu	Pro Phe Val	Pro Pro Thr	GG1 L235 Cys Pro 25 Cys	Pro 10 Lys Val	Ala Pro Val	Pro Lys Val	T36 Glu Asp Asp 45	Ala Thr 30 Val	Ala 15 Leu Ser	ions Gly Met His
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<211 <212 <213 <220 <400 <400 Gly Ile Glu His 65	> LE > TY > OF > OF > OT > OT	ENGTHER COLUMN THE COL	H: 22 PRT SM: RE: INFO pptic UCE: His Thr Glu Lys	Arti DRMAT de wi 68 Thr 5 Phe Pro Val	rion: ith a Cys Leu Glu Lys	Pro Phe Val Pro Phe Fro	Pro Pro Thr 40 Asn	gG1 L235 Cys Pro 25 Cys Trp	Pro 10 Lys Val Tyr	Ala Pro Val Val Gln 75	Pro Lys Val Asp 60	T36 Glu Asp Asp 45 Gly Asn	Ala Thr 30 Val Val Ser	Ala 15 Leu Ser Glu	Gly Met His Val Tyr
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	130					135					140				
Leu 145	Trp	Cys	Leu	Val	Lys 150	Gly	Phe	Tyr	Pro	Ser 155	Asp	Ile	Ala	Val	Glu 160
Trp	Glu	Ser	Asn	Gly 165	Gln	Pro	Glu	Asn	Asn 170	Tyr	ГÀа	Thr	Thr	Pro 175	Pro
Val	Leu	Asp	Ser 180	Asp	Gly	Ser	Phe	Phe 185	Leu	Tyr	Ser	Lys	Leu 190	Thr	Val
Asp	Lys	Ser 195	Arg	Trp	Gln	Gln	Gly 200	Asn	Val	Phe	Ser	Сув 205	Ser	Val	Met
His	Glu 210	Ala	Leu	His	Asn	His 215	Tyr	Thr	Gln	Lys	Ser 220	Leu	Ser	Leu	Ser
Pro 225	Gly	Lys													
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Asp 1	ГЛа	Thr	His	Thr 5	CAa	Pro	Pro	CAa	Pro 10	Ala	Pro	Glu	Leu	Leu 15	Gly
Gly	Pro	Ser	Val 20	Phe	Leu	Phe	Pro	Pro 25	Lys	Pro	Lys	Asp	Thr 30	Leu	Met
Ile	Ser	Arg 35	Thr	Pro	Glu	Val	Thr 40	Cys	Val	Val	Val	Asp 45	Val	Ser	His
Glu	Asp 50	Pro	Glu	Val	Lys	Phe 55	Asn	Trp	Tyr	Val	Asp 60	Gly	Val	Glu	Val
His 65	Asn	Ala	Lys	Thr	Lys 70	Pro	Arg	Glu	Glu	Gln 75	Tyr	Asn	Ser	Thr	Tyr 80
Arg	Val	Val	Ser	Val 85	Leu	Thr	Val	Leu	His 90	Gln	Asp	Trp	Leu	Asn 95	Gly
Lys	Glu	Tyr	Tys	CÀa	rya	Val	Ser	Asn 105	Lys	Ala	Leu	Gly	Ala 110	Pro	Ile
Glu	Lys	Thr 115	Ile	Ser	Lys	Ala	Lys 120	Gly	Gln	Pro	Arg	Glu 125	Pro	Gln	Val
Tyr	Thr 130	Leu	Pro	Pro	Ser	Arg 135	Asp	Glu	Leu	Thr	Lys 140	Asn	Gln	Val	Ser
Leu 145	Thr	СЛа	Leu	Val	Lys 150	Gly	Phe	Tyr	Pro	Ser 155	Asp	Ile	Ala	Val	Glu 160
Trp	Glu	Ser	Asn	Gly 165	Gln	Pro	Glu	Asn	Asn 170	Tyr	Lys	Thr	Thr	Pro 175	Pro
Val	Leu	Asp	Ser 180	Asp	Gly	Ser	Phe	Phe 185	Leu	Tyr	Ser	Lys	Leu 190	Thr	Val
Asp	Lys	Ser 195	Arg	Trp	Gln	Gln	Gly 200	Asn	Val	Phe	Ser	Cys 205	Ser	Val	Met
His	Glu 210	Ala	Leu	His	Asn	His 215	Tyr	Thr	Gln	Lys	Ser 220	Leu	Ser	Leu	Ser
Pro 225	Gly	Lys													

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human IgG1 Fc-region derived Fc-region
    polypeptide with L234A, L235A mutations and P329G mutation
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Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
               120
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
  130 135
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
                 150
                                   155
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                       170
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
          180
                   185
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
     195 200
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
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Pro Gly Lys
<210> SEQ ID NO 71
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human IgG1 Fc-region derived Fc-region
     polypeptide with a P239G mutation and Y349C, T366S, L368A, Y407V
     mutations
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Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
                     25
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
               40
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Glu Asp Pro	Glu	Val	Lys	Phe 55	Asn	Trp	Tyr	Val	Asp 60	Gly	Val	Glu	Val	
His Asn Ala 65	Lys	Thr	Lys 70	Pro	Arg	Glu	Glu	Gln 75	Tyr	Asn	Ser	Thr	Tyr 80	
Arg Val Val	Ser	Val 85	Leu	Thr	Val	Leu	His 90	Gln	Asp	Trp	Leu	Asn 95	Gly	
Lys Glu Tyr	Lys 100	СЛа	Lys	Val	Ser	Asn 105	ГЛа	Ala	Leu	Gly	Ala 110	Pro	Ile	
Glu Lys Thr 115	Ile	Ser	Lys	Ala	Lys 120	Gly	Gln	Pro	Arg	Glu 125	Pro	Gln	Val	
Cys Thr Leu 130	Pro	Pro	Ser	Arg 135	Asp	Glu	Leu	Thr	Lys 140	Asn	Gln	Val	Ser	
Leu Ser Cys 145	Ala	Val	Lys 150	Gly	Phe	Tyr	Pro	Ser 155	Asp	Ile	Ala	Val	Glu 160	
Trp Glu Ser	Asn	Gly 165	Gln	Pro	Glu	Asn	Asn 170	Tyr	ГÀа	Thr	Thr	Pro 175	Pro	
Val Leu Asp	Ser 180	Asp	Gly	Ser	Phe	Phe 185	Leu	Val	Ser	ГÀа	Leu 190	Thr	Val	
Asp Lys Ser 195	Arg	Trp	Gln	Gln	Gly 200	Asn	Val	Phe	Ser	Сув 205	Ser	Val	Met	
His Glu Ala 210	Leu	His	Asn	His 215	Tyr	Thr	Gln	Lys	Ser 220	Leu	Ser	Leu	Ser	
Pro Gly Lys 225														
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145															
165	145				150					155					160
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met 210	Trp Glu	Ser	Asn	_	Gln	Pro	Glu	Asn		Tyr	Lys	Thr	Thr		Pro
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser 210 Sep Cly Lys 225 **C210 SEQ ID NO 73	Val Leu	Asp		Asp	Gly	Ser	Phe		Leu	Tyr	Ser	Lys		Thr	Val
210 215	Asp Lys		Arg	Trp	Gln	Gln	_	Asn	Val	Phe	Ser	_	Ser	Val	Met
2210 SEQ ID NO 73 <2111		Ala	Leu	His	Asn		Tyr	Thr	Gln	Lys		Leu	Ser	Leu	Ser
<pre> 2212</pre>	-	Lys													
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Lys Pro Lys Asp Thr Leu Met 15 Gly Pro Ser Val Phe Leu Phe Pro 25 Lys Pro Lys Asp Thr Leu Met 30 Ile Ser Arg Thr Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val Cys Asp Thr Roman Ser Thr Tyr Tyr Val Asp Gly Val Glu Val Cys Asp Thr Roman Ser Thr Tyr Tyr Val Asp Gly Val Glu Val Cys Roman Ser Thr Tyr Tyr Val Asp Gly Val Glu Val Cys Roman Ser Thr Tyr Tyr Val Asp Gly Val Glu Val Cys Roman Ser Thr Tyr Pro Roman Ser T	<211> LI <212> TY <213> OF <220> FI <223> OF YA	ENGTI YPE: RGAN: EATUI THER Olype 407V	H: 2: PRT ISM: RE: INFO ptic mut	Art ORMA de w atio	TION ith	: hui	man :	IgG1							
16		_													
Ser		Thr	His		Cya	Pro	Pro	Cya		Ala	Pro	Glu	Ala		Gly
Second S	Gly Pro	Ser		Phe	Leu	Phe	Pro		Lys	Pro	Lys	Asp		Leu	Met
Fig.	Ile Ser	_	Thr	Pro	Glu	Val		Cys	Val	Val	Val	_	Val	Ser	His
65 70 75 80 Arg Val Val Val Ser Val Ser Val Ser Val Ser Nam Val Leu His Son Glu Ser Glu Pro Ser Into 100 1 Leu Ser Val Val Ser Ash Lys Ala Leu Gly Ala Pro Into 110 1 Leu Ser Lys Ala Lys Glu Pro Arg Glu Pro Glu Val 110 1 Leu Ser Clu Pro Glu		Pro	Glu	Val	Lys		Asn	Trp	Tyr	Val		Gly	Val	Glu	Val
Second S		Ala	Lys	Thr		Pro	Arg	Glu	Glu		Tyr	Asn	Ser	Thr	
Ser Ser Ser Asp Ser Asp Ser	Arg Val	Val	Ser		Leu	Thr	Val	Leu		Gln	Asp	Trp	Leu		Gly
115	Lys Glu	Tyr	_	Cys	Lys	Val	Ser		Lys	Ala	Leu	Gly		Pro	Ile
130 135 140 Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu 160 Trp Glu Ser Asp Gly Gln Pro Glu Asp Asp Tyr Lys Thr Thr Pro Pro 175 Val Leu Asp Ser Asp Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val 180 Asp Lys Ser Arg Trp Gln Gln Gly Asp Val Phe Ser Cys Ser Val Met 210 His Glu Ala Leu His Asp His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys	Glu Lys		Ile	Ser	Lys	Ala		Gly	Gln	Pro	Arg		Pro	Gln	Val
145 150 155 160 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro 175 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val 180 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met 210 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser 210 Pro Gly Lys	_	Leu	Pro	Pro	Ser	_	Asp	Glu	Leu	Thr	_	Asn	Gln	Val	Ser
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Asp Asp His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Lys Lys Lys Lys Lys Lys		CÀa	Ala	Val	-	_	Phe	Tyr	Pro		Asp	Ile	Ala	Val	
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met 195	Trp Glu	Ser	Asn	_	Gln	Pro	Glu	Asn		Tyr	Lys	Thr	Thr		Pro
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser 210 Pro Gly Lys	Val Leu	Asp		Asp	Gly	Ser	Phe		Leu	Val	Ser	ГÀа		Thr	Val
210 215 220 Pro Gly Lys	Asp Lys		Arg	Trp	Gln	Gln	_	Asn	Val	Phe	Ser	_	Ser	Val	Met
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		ГÀа													

<211> LENGTH: 227

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<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human IgG1 Fc-region derived Fc-region
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      T366W mutations
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Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
       55 60
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile
                             105
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
                       120
Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
                     135
Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                     170
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
         180 185
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                         200
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
Pro Gly Lys
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<210> SEQ ID NO 75
<211> LENGTH: 229
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human IgG4 Fc-region derived Fc-region
     polypeptide with S228P and L235E mutations
<400> SEQUENCE: 75
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe
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Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
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	50					55					60				
Glu 65	Val	His	Asn	Ala	Lys 70	Thr	Lys	Pro	Arg	Glu 75	Glu	Gln	Phe	Asn	Ser 80
Thr	Tyr	Arg	Val	Val 85	Ser	Val	Leu	Thr	Val 90	Leu	His	Gln	Asp	Trp 95	Leu
Asn	Gly	Lys	Glu 100	Tyr	Lys	Cys	Lys	Val 105	Ser	Asn	Lys	Gly	Leu 110	Pro	Ser
Ser	Ile	Glu 115	Lys	Thr	Ile	Ser	Lys 120	Ala	Lys	Gly	Gln	Pro 125	Arg	Glu	Pro
Gln	Val 130	Tyr	Thr	Leu	Pro	Pro 135	Ser	Gln	Glu	Glu	Met 140	Thr	Lys	Asn	Gln
Val 145	Ser	Leu	Thr	CÀa	Leu 150	Val	Lys	Gly	Phe	Tyr 155	Pro	Ser	Asp	Ile	Ala 160
Val	Glu	Trp	Glu	Ser 165	Asn	Gly	Gln	Pro	Glu 170	Asn	Asn	Tyr	Lys	Thr 175	Thr
Pro	Pro	Val	Leu 180	Asp	Ser	Asp	Gly	Ser 185	Phe	Phe	Leu	Tyr	Ser 190	Arg	Leu
Thr	Val	Asp 195	Lys	Ser	Arg	Trp	Gln 200	Glu	Gly	Asn	Val	Phe 205	Ser	Cys	Ser
Val	Met 210	His	Glu	Ala	Leu	His 215	Asn	His	Tyr	Thr	Gln 220	Lys	Ser	Leu	Ser
Leu 225	Ser	Leu	Gly	ГÀа											
<220 <223)> FE 3> OI po	EATUR THER Dlype	RE: INFO		TION	: hur	nan]	[gG4							gion ition
)> SE Ser	-		76 Gly	Pro	Pro	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Phe
1 Glu	Gly	Gly		5 Ser	Val	Phe	Leu		10 Pro	Pro	Lys	Pro		15 Asp	Thr
Leu	Met		20 Ser	Arg	Thr	Pro		25 Val	Thr	Cys	Val		30 Val	Asp	Val
Ser		35 Glu	Asp	Pro	Glu		40 Gln	Phe	Asn	Trp		45 Val	Asp	Gly	Val
	50 Val	His	Asn	Ala		55 Thr	Lys	Pro	Arg		60 Glu	Gln	Phe	Asn	
65															
Thr	Tyr	Arg	Val	Val	70 Ser	Val	Leu	Thr		75 Leu	His	Gln	Asp	_	80 Leu
	-		Glu	Val 85 Tyr	Ser			Val	90	Leu			Leu	95	Leu
Asn	Gly	Lys	Glu 100	85	Ser Lys	Сув	Lys	Val 105	90 Ser	Leu Asn	Lys	Gly	Leu 110	95 Gly	Leu Ser
Asn Ser	Gly	Lys Glu 115	Glu 100 Lys	85 Tyr	Ser Lys Ile	Cys Ser	Lys Lys 120	Val 105 Ala	90 Ser Lys	Leu Asn Gly	Lys Gln	Gly Pro 125	Leu 110 Arg	95 Gly Glu	Leu Ser Pro
Asn Ser Gln	Gly Ile Val	Lys Glu 115 Tyr	Glu 100 Lys Thr	85 Tyr Thr	Ser Lys Ile Pro	Cys Ser Pro 135	Lys Lys 120 Ser	Val 105 Ala Gln	90 Ser Lys Glu	Leu Asn Gly Glu	Lys Gln Met 140	Gly Pro 125 Thr	Leu 110 Arg Lys	95 Gly Glu Asn	Leu Ser Pro Gln

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Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
                    185
Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
Leu Ser Leu Gly Lys
<210> SEQ ID NO 77
<211> LENGTH: 229
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human IgG4 Fc-region derived Fc-region
    polypeptide with S354C, T366W mutations
<400> SEQUENCE: 77
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                40
Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
                              105
Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
Gln Val Tyr Thr Leu Pro Pro Cys Gln Glu Glu Met Thr Lys Asn Gln
Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
  210 215
Leu Ser Leu Gly Lys
225
<210> SEQ ID NO 78
<211> LENGTH: 229
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: human IgG4 Fc-region derived Fc-region
     polypeptide with Y349C, T366S, L368A, Y407V mutations
<400> SEQUENCE: 78
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
65 70 75 80
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
      115 120
Gln Val Cys Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
        135
Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                       170
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Arg Leu
                            185
Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
                           200
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
                      215
Leu Ser Leu Gly Lys
225
<210> SEQ ID NO 79
<211> LENGTH: 229
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human IgG4 Fc-region derived Fc-region
     polypeptide with a S228P, L235E and S354C, T366W mutations
<400> SEQUENCE: 79
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe
Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                    25
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                         40
Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
                     55
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
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Thr	Tyr	Arg	Val	Val 85	Ser	Val	Leu	Thr	Val 90	Leu	His	Gln	Asp	Trp 95	Leu
Asn	Gly	Lys	Glu 100	Tyr	Lys	CÀa	Lys	Val 105	Ser	Asn	Lys	Gly	Leu 110	Pro	Ser
Ser	Ile	Glu 115	Lys	Thr	Ile	Ser	Lys 120	Ala	ГЛа	Gly	Gln	Pro 125	Arg	Glu	Pro
Gln	Val 130	Tyr	Thr	Leu	Pro	Pro 135	Cys	Gln	Glu	Glu	Met 140	Thr	Lys	Asn	Gln
Val 145	Ser	Leu	Trp	CAa	Leu 150	Val	Lys	Gly	Phe	Tyr 155	Pro	Ser	Asp	Ile	Ala 160
Val	Glu	Trp	Glu	Ser 165	Asn	Gly	Gln	Pro	Glu 170	Asn	Asn	Tyr	Lys	Thr 175	Thr
Pro	Pro	Val	Leu 180	Asp	Ser	Asp	Gly	Ser 185	Phe	Phe	Leu	Tyr	Ser 190	Arg	Leu
Thr	Val	Asp 195	Lys	Ser	Arg	Trp	Gln 200	Glu	Gly	Asn	Val	Phe 205	Ser	Cys	Ser
Val	Met 210	His	Glu	Ala	Leu	His 215	Asn	His	Tyr	Thr	Gln 220	rys	Ser	Leu	Ser
Leu 225	Ser	Leu	Gly	ГЛа											
<213 <220	2 > T\ 3 > OF 3 > FE 3 > OT	RGANI EATUF	SM: RE:				-		Pa-x	-oale	n de	rive	ad Ea		
	pc	lyp∈ 107V	eptid muta	le wi tior	ith a										
<400	po Y4	olype 107V EQUEN	eptic muta ICE:	le wi stion 80	ith a	a S22	28P,	L235	E an	nd Y3	349C,	T36	56S,	L368	BA,
<400 Glu 1	po Y4)> SE	olype 107V EQUEN Lys	eptic muta ICE: Tyr	le wi tior 80 Gly 5	ith a	R S22	Cys	L235	E ar Pro 10	cys	949C,	T36	Pro	L368 Glu 15	BA, Phe
<400 Glu 1 Glu	po Y4)> SE Ser	olype 107V EQUEN Lys Gly	eptic muta JCE: Tyr Pro 20	de wi atior 80 Gly 5 Ser	ith ans Pro Val	Pro	Cys Leu	Pro Phe	Pro 10	Cys Pro	Pro Lys	T36	Pro Lys 30	Glu 15 Asp	Phe Thr
<400 Glu 1 Glu Leu	po Y4)> SE Ser Gly	olype 107V EQUEN Lys Gly Ile 35	eptic muta JCE: Tyr Pro 20	de wi atior 80 Gly 5 Ser Arg	ith ans Pro Val	Pro Phe Pro	Cys Leu Glu 40	Pro Phe 25 Val	Pro 10 Pro Thr	Cys Pro Cys	Pro Lys Val	T36 Ala Pro Val 45	Pro Lys 30 Val	Glu 15 Asp	Phe Thr
<400 Glu 1 Glu Leu Ser	po Y4)> SE Ser Gly Met	olype 107V EQUEN Lys Gly Ile 35	eptic muta JCE: Tyr Pro 20 Ser Asp	de wition 80 Gly 5 Ser Arg Pro	tth ans Pro Val Thr	Pro Phe Pro Val 55	Cys Leu Glu 40 Gln Lys	Pro Phe 25 Val Phe Pro	Pro 10 Pro Thr Asn	Cys Pro Cys Trp Glu	Pro Lys Val Tyr 60	T36 Ala Pro Val 45 Val	Pro Lys 30 Val	Glu 15 Asp Asp	Phe Thr Val
<400 Glu 1 Glu Leu Ser Glu 65	pc Y4 Ser Gly Met Gln 50	olype 107V CQUEN Lys Gly Ile 35 Glu	eptic muta JCE: Tyr Pro 20 Ser Asp	de wition 80 Gly 5 Ser Arg Pro	th ans Pro Val Thr Glu Lys	Pro Phe Pro Val 55	Cys Leu Glu 40 Gln Lys	Pro Phe 25 Val Phe	Pro 10 Pro Thr Asn	nd Y2 Cys Pro Cys Trp Glu 75	Pro Lys Val Tyr 60	Ala Pro Val 45 Val Gln	Pro Lys 30 Val Asp	Glu 15 Asp Asp Gly	Phe Thr Val Val Ser
<400 Glu 1 Glu Leu Ser Glu 65 Thr	po Y44 Ser Ser Gly Met Gln 50 Val	olype 107V Lys Gly Ile 35 Glu His	eptic muta JCE: Tyr Pro 20 Ser Asp Asn	de wittion 80 Gly 5 Ser Arg Pro Ala Val 85	Pro Val Thr Glu Lys 70 Ser	Pro Phe Pro Val 55 Thr	Cys Leu Glu 40 Gln Lys Leu	Pro Phe 25 Val Phe Thr	Pro 10 Pro Thr Asn Arg Val	Cys Pro Cys Trp Glu 75 Leu	Pro Lys Val Tyr 60 Glu	Ala Pro Val 45 Val Gln	Pro Lys 30 Val Asp Phe Asp	Glu 15 Asp Asp Gly Asn Trp 95	Phe Thr Val Val Ser 80 Leu
<4000 Glu 1 Glu Leu Ser Glu 65 Thr	pc Y4 Ser Ser Gly Met Gln 50 Val	lype 107V CQUEN Lys Gly Ile 35 Glu His Arg	eptic muta JCE: Tyr Pro 20 Ser Asp Asn Val Glu 100	de wittion 80 Gly 5 Ser Arg Pro Ala Val 85 Tyr	Pro Val Thr Glu Lys 70 Ser	Pro Phe Pro Val 55 Thr Val	Cys Leu Glu 40 Gln Lys Leu	Pro Phe 25 Val Phe Pro Thr Val	Pro 10 Pro Thr Asn Arg Val 90 Ser	Cys Pro Cys Trp Glu 75 Leu Asn	Pro Lys Val Tyr 60 Glu His	Ala Pro Val 45 Val Gln Gly	Pro Lys 30 Val Asp Phe Asp Leu 110	Glu 15 Asp Asp Gly Asn Trp 95 Pro	Phe Thr Val Val Ser 80 Leu Ser
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<400 Glu 1 Glu Leu Ser Glu 65 Thr Asn Ser Gln	po y/4 D> SE Ser Gly Met Gln 50 Val Tyr Gly Ile Val	olype 107V EQUEN Lys Gly Ile 35 Glu His Arg Lys Glu 115 Cys	muta muta Tyr Pro 20 Ser Asp Asn Val Glu 100 Lys	de wittion 80 Gly 5 Ser Arg Pro Ala Val 85 Tyr Thr	Pro Val Thr Glu Lys 70 Ser Lys Ile	Pro Phe Pro Val 55 Thr Val Cys Ser	Cys Leu Glu 40 Gln Lys Leu Lys Ser	Pro Phe 25 Val Phe Pro Thr Val 105 Ala Gln	Pro 10 Pro Thr Asn Arg Val 90 Ser Lys	Cys Pro Cys Trp Glu 75 Leu Asn Gly Glu	Pro Lys Val Tyr 60 Glu His Lys Gln Met 140	Ala Pro Val 45 Val Gln Gln Gly Pro 125	Pro Lys 30 Val Asp Phe Asp Leu 110 Arg	Glu 15 Asp Asp Gly Asn Trp 95 Pro Glu Asn	Phe Thr Val Val Ser 80 Leu Ser Pro Gln

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Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Arg Leu
                              185
Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
                200
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
Leu Ser Leu Gly Lys
<210> SEQ ID NO 81
<211> LENGTH: 229
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human IgG4 Fc-region derived Fc-region
    polypeptide with a P329G mutation
<400> SEQUENCE: 81
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
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Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                              25
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
              85
                                  90
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Gly Ser
Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
               120
Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
           135
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
                        200
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
                      215
                                          220
Leu Ser Leu Gly Lys
225
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<211> LENGTH: 229
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human IgG4 Fc-region derived Fc-region
    polypeptide with a P239G and Y349C, T366S, L368A, Y407V
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mutations
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Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val 50 60
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu 85 90 95
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Gly Ser
                   105
          100
Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
                          120
Gln Val Cys Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
                      135
Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
                150
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                                  170
              165
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Arg Leu
Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
                200
                                      205
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
                      215
Leu Ser Leu Gly Lys
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<210> SEQ ID NO 83
<211> LENGTH: 229
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human IgG4 Fc-region derived Fc-region
    polypeptide with a P329G and S354C, T366W mutations
<400> SEQUENCE: 83
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe 1 5 10 15
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                               25
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                40
Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
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				85					90					95	
Asn	Gly	Lys	Glu 100	Tyr	Lys	Cys	Lys	Val 105	Ser	Asn	Lys	Gly	Leu 110	Gly	Ser
Ser	Ile	Glu 115	Lys	Thr	Ile	Ser	Lys 120	Ala	Lys	Gly	Gln	Pro 125	Arg	Glu	Pro
Gln	Val 130	Tyr	Thr	Leu	Pro	Pro 135	Сув	Gln	Glu	Glu	Met 140	Thr	Lys	Asn	Gln
Val 145	Ser	Leu	Trp	CÀa	Leu 150	Val	Lys	Gly	Phe	Tyr 155	Pro	Ser	Asp	Ile	Ala 160
Val	Glu	Trp	Glu	Ser 165	Asn	Gly	Gln	Pro	Glu 170	Asn	Asn	Tyr	Lys	Thr 175	Thr
Pro	Pro	Val	Leu 180	Asp	Ser	Asp	Gly	Ser 185	Phe	Phe	Leu	Tyr	Ser 190	Arg	Leu
Thr	Val	Asp 195	ГÀа	Ser	Arg	Trp	Gln 200	Glu	Gly	Asn	Val	Phe 205	Ser	CÀa	Ser
Val	Met 210	His	Glu	Ala	Leu	His 215	Asn	His	Tyr	Thr	Gln 220	ГÀа	Ser	Leu	Ser
Leu 225	Ser	Leu	Gly	ГАв											
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Glu	Gly	Gly	Pro 20	Ser	Val	Phe	Leu	Phe 25	Pro	Pro	Lys	Pro	Lys 30	Asp	Thr
Leu	Met	Ile 35	Ser	Arg	Thr	Pro	Glu 40	Val	Thr	Сув	Val	Val 45	Val	Asp	Val
Ser	Gln 50	Glu	Asp	Pro	Glu	Val 55	Gln	Phe	Asn	Trp	Tyr 60	Val	Asp	Gly	Val
Glu 65	Val	His	Asn	Ala	Lys 70	Thr	Lys	Pro	Arg	Glu 75	Glu	Gln	Phe	Asn	Ser 80
Thr	Tyr	Arg	Val	Val 85	Ser	Val	Leu	Thr	Val 90	Leu	His	Gln	Asp	Trp 95	Leu
Asn	Gly	Lys	Glu 100	Tyr	ГÀа	Cys	Lys	Val 105	Ser	Asn	ГÀа	Gly	Leu 110	Gly	Ser
Ser	Ile	Glu 115	Lys	Thr	Ile	Ser	Lys 120	Ala	Lys	Gly	Gln	Pro 125	Arg	Glu	Pro
Gln	Val 130	Сув	Thr	Leu	Pro	Pro 135	Ser	Gln	Glu	Glu	Met 140	Thr	Lys	Asn	Gln
Val 145	Ser	Leu	Ser	CÀa	Ala 150	Val	Lys	Gly	Phe	Tyr 155	Pro	Ser	Asp	Ile	Ala 160
Val	Glu	Trp	Glu	Ser 165	Asn	Gly	Gln	Pro	Glu 170	Asn	Asn	Tyr	Lys	Thr 175	Thr
Pro	Pro	Val	Leu 180	Asp	Ser	Asp	Gly	Ser 185	Phe	Phe	Leu	Val	Ser 190	Arg	Leu

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Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr 20 25 30
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
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Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Gly Ser
                 105
Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
               120
Gln Val Tyr Thr Leu Pro Pro Cys Gln Glu Glu Met Thr Lys Asn Gln
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Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
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Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
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Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
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50	Tyr	Pro	•	Ala	Val	Thr	Val		Trp	Lys	Ala	Asp		Ser	Pro	Val
## Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Ser Thr Val Glu Ser Thr Val Ala Pro Thr Glu Cys Ser 100	Lys		Gly	Val	Glu	Thr		Thr	Pro	Ser	Lys		Ser	Asn	Asn	Lys
Lys Thr Val Ala Pro Thr Glu Cys Ser 100		Ala	Ala	Ser	Ser		Leu	Ser	Leu	Thr		Glu	Gln	Trp	Lys	
100 105	His	Arg	Ser	Tyr		CÀa	Gln	Val	Thr		Glu	Gly	Ser	Thr		Glu
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Arg Thr Val Ala Ala Pro Ser Val Phe 11e Phe Pro Pro Ser Asp Glu 1						sa]	piens	3								
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Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Sor Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu 80 Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser 90 Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys 100 <pre></pre>	Gln	Leu	Lys		Gly	Thr	Ala	Ser		Val	Cys	Leu	Leu		Asn	Phe
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65	Ser		Asn	Ser	Gln	Glu		Val	Thr	Glu	Gln		Ser	Lys	Asp	Ser
Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys		Tyr	Ser	Leu	Ser		Thr	Leu	Thr	Leu		Lys	Ala	Asp	Tyr	
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Tyr Asp Ala Ser Lys Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly 50 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro 65 Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Lys Trp Pro Pro	Glu	Arg	Ala		Leu	Ser	CÀa	Arg		Ser	Gln	Ser	Val		Ser	Tyr
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro 75 80 Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Lys Trp Pro Pro	Leu	Ala	_	Tyr	Gln	Gln	ГЛа		Gly	Gln	Ala	Pro	_	Leu	Leu	Ile
65 70 75 80 Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Lys Trp Pro Pro	Tyr	_	Ala	Ser	Lys	Arg		Thr	Gly	Ile	Pro		Arg	Phe	Ser	Gly
		Gly	Ser	Gly	Thr		Phe	Thr	Leu	Thr		Ser	Ser	Leu	Glu	
	Glu	Asp	Phe	Ala		Tyr	Tyr	Сув	Gln		Arg	Ser	Lys	Trp		Pro

Trp	Thr	Phe	Gly 100	Gln	Gly	Thr	Lys	Val 105	Glu	Ser	Lys	Arg	Thr 110	Val	Ala
Ala	Pro	Ser 115	Val	Phe	Ile	Phe	Pro 120	Pro	Ser	Asp	Glu	Gln 125	Leu	Lys	Ser
Gly	Thr 130	Ala	Ser	Val	Val	Сув 135	Leu	Leu	Asn	Asn	Phe 140	Tyr	Pro	Arg	Glu
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Gln	Glu	Ser	Val	Thr 165	Glu	Gln	Asp	Ser	Lys 170	Asp	Ser	Thr	Tyr	Ser 175	Leu
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Tyr	Ala	Сув 195	Glu	Val	Thr	His	Gln 200	Gly	Leu	Ser	Ser	Pro 205	Val	Thr	Lys
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Ala	Ile 50	Ile	Trp	Phe	Asp	Gly 55	Ser	Ser	Thr	Tyr	Tyr 60	Ala	Asp	Ser	Val
Arg 65	Gly	Arg	Phe	Thr	Ile 70	Ser	Arg	Asp	Asn	Ser 75	ràs	Asn	Thr	Leu	Tyr 80
Leu	Gln	Met	Asn	Ser 85	Leu	Arg	Ala	Glu	Asp 90	Thr	Ala	Val	Tyr	Phe 95	Cys
Ala	Arg	Glu	Leu 100	Gly	Arg	Arg	Tyr	Phe 105	Asp	Leu	Trp	Gly	Arg 110	Gly	Thr
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Leu	Ala 130	Pro	Ser	Ser	TÀa	Ser 135	Thr	Ser	Gly	Gly	Thr 140	Ala	Ala	Leu	Gly
Cys 145	Leu	Val	TÀS	Aap	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Ser	Trp	Asn 160
Ser	Gly	Ala	Leu	Thr 165	Ser	Gly	Val	His	Thr 170	Phe	Pro	Ala	Val	Leu 175	Gln
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Ser	Leu	Gly 195	Thr	Gln	Thr	Tyr	Ile 200	Cys	Asn	Val	Asn	His 205	Lys	Pro	Ser
Asn	Thr 210	Lys	Val	Asp	Lys	Lys 215	Val	Glu	Pro	Lys	Ser 220	CAa	Asp	Lys	Thr

225	Pro Pro	Cys Pro 230	Ala Pro	Glu Leu 235	Leu Gly	Gly Pro	Ser 240
Val Phe Leu	Phe Pro		Pro Lys	Asp Thr 250	Leu Met	: Ile Se: 25!	
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Glu Val Lys 275	Phe Asn	Trp Tyr	Val Asp 280	Gly Val	Glu Val 285		n Ala
Lys Thr Lys 290	Pro Arg	Glu Glu 295		Asn Ser	Thr Tyr	Arg Val	l Val
Ser Val Leu 305	Thr Val	Leu His 310	Gln Asp	Trp Leu 315	Asn Gly	' Lys Gl	1 Tyr 320
Lya Cya Lya	Val Ser 325	_	Ala Leu	Pro Ala 330	Pro Ile	Glu Ly: 33!	
Ile Ser Lys	Ala Lys 340	Gly Gln	Pro Arg 345		Gln Val	. Tyr Th: 350	r Leu
Pro Pro Ser 355	Arg Asp	Glu Leu	Thr Lys	Asn Gln	Val Ser 365		r Cha
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Asn Gly Gln 385	Pro Glu	Asn Asn 390	Tyr Lys	Thr Thr 395	Pro Pro	Val Le	1 Asp 400
Ser Asp Gly	Ser Phe 405	Phe Leu	Tyr Ser	Lys Leu 410	Thr Val	. Asp Ly: 41!	
Arg Trp Gln	Gln Gly 420	Asn Val	Phe Ser 425		Val Met	His Glu	ı Ala
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Ser Gln Arg	5 Leu Ser 20 Trp Val	Cys Ala	Ala Ser 25 Ala Pro 40	Gly Phe	Thr Phe	15 Ser Se: 30 Glu Tr	r Tyr o Val
Ser Gln Arg Gly Met His 35 Ala Ile Ile	5 Leu Ser 20 Trp Val Trp Phe	Cys Ala Arg Gln Asp Gly	Ala Ser 25 Ala Pro 40 Ser Ser	Gly Phe Gly Lys Thr Tyr	Thr Phe Gly Leu 45 Tyr Ala	15 Ser Se: 30 Glu Tr	r Tyr O Val
Ser Gln Arg Gly Met His 35 Ala Ile Ile 50 Arg Gly Arg	5 Leu Ser 20 Trp Val Trp Phe Phe Thr	Cys Ala Arg Gln Asp Gly 55 Ile Ser 70	Ala Ser 25 Ala Pro 40 Ser Ser Arg Asp	Gly Phe Gly Lys Thr Tyr Asn Ser	Thr Phe Gly Leu 45 Tyr Ala 60 Lys Asr	15 Ser Ser 30 Try Asp Ser	r Tyr Val Val Tyr 80
Ser Gln Arg Gly Met His 35 Ala Ile Ile 50 Arg Gly Arg 65	5 Leu Ser 20 Trp Val Trp Phe Phe Thr Asn Ser 85	Cys Ala Arg Gln Asp Gly 55 Ile Ser 70 Leu Arg	Ala Ser 25 Ala Pro 40 Ser Ser Arg Asp	Gly Phe Gly Lys Thr Tyr Asn Ser 75 Asp Thr 90 Asp Leu	Thr Phe Gly Let 45 Tyr Ala 60 Lys Asr	15 Ser Se: 30 Trp a Asp Se: a Thr Let 5 Tyr Pho	r Tyr Val Val Tyr 80 Cys
Ser Gln Arg Gly Met His 35 Ala Ile Ile 50 Arg Gly Arg 65 Leu Gln Met	Leu Ser 20 Trp Val Trp Phe Phe Thr Asn Ser 85 Leu Gly 100	Cys Ala Arg Gln Asp Gly 55 Ile Ser 70 Leu Arg Arg Arg	Ala Ser 25 Ala Pro 40 Ser Ser Arg Asp Ala Glu Tyr Phe 105	Gly Phe Gly Lys Thr Tyr Asn Ser 75 Asp Thr 90 Asp Leu	Thr Phe Gly Let 45 Tyr Ala 60 Lys Asr Ala Val	15 Ser Ser 30 Try Pho 95 Arg Glr 110 Tval Pho 110 Try	r Tyr Val Val Tyr 80 Cys

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly 135 Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser 225 230 235 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ala Ser Arg 250 245 Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro 265 Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala 280 Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val 295 Ser Val Leu Thr Val Leu Ala Gln Asp Trp Leu Asn Gly Lys Glu Tyr 310 315 Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu 345 Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys 360 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser 375 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Ala Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly <210> SEQ ID NO 91 <211> LENGTH: 447 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223 > OTHER INFORMATION: IGF-1R YTE <400> SEQUENCE: 91 Gln Val Glu Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg 10

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Ala	Ile 50	Ile	Trp	Phe	Asp	Gly 55	Ser	Ser	Thr	Tyr	Tyr 60	Ala	Asp	Ser	Val
Arg 65	Gly	Arg	Phe	Thr	Ile 70	Ser	Arg	Asp	Asn	Ser 75	Lys	Asn	Thr	Leu	Tyr 80
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Ala	Arg	Glu	Leu 100	Gly	Arg	Arg	Tyr	Phe 105	Asp	Leu	Trp	Gly	Arg 110	Gly	Thr
Leu	Val	Ser 115	Val	Ser	Ser	Ala	Ser 120	Thr	Lys	Gly	Pro	Ser 125	Val	Phe	Pro
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Ser	Gly	Ala	Leu	Thr 165	Ser	Gly	Val	His	Thr 170	Phe	Pro	Ala	Val	Leu 175	Gln
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Ser	Leu	Gly 195	Thr	Gln	Thr	Tyr	Ile 200	Cys	Asn	Val	Asn	His 205	ГЛа	Pro	Ser
Asn	Thr 210	Lys	Val	Asp	Lys	Lys 215	Val	Glu	Pro	Lys	Ser 220	CÀa	Asp	Lys	Thr
His 225	Thr	Cys	Pro	Pro	Сув 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240
Val	Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp 250	Thr	Leu	Tyr	Ile	Thr 255	Arg
Glu	Pro	Glu	Val 260	Thr	Сув	Val	Val	Val 265	Asp	Val	Ser	His	Glu 270	Asp	Pro
Glu	Val	Lys 275	Phe	Asn	Trp	Tyr	Val 280	Asp	Gly	Val	Glu	Val 285	His	Asn	Ala
Lys	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val
Ser 305	Val	Leu	Thr	Val	Leu 310	His	Gln	Asp	Trp	Leu 315	Asn	Gly	Lys	Glu	Tyr 320
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Ile	Ser	Lys	Ala 340	Lys	Gly	Gln	Pro	Arg 345	Glu	Pro	Gln	Val	Tyr 350	Thr	Leu
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Leu	Val 370	Lys	Gly	Phe	Tyr	Pro 375	Ser	Asp	Ile	Ala	Val 380	Glu	Trp	Glu	Ser
Asn 385	Gly	Gln	Pro	Glu	Asn 390	Asn	Tyr	Lys	Thr	Thr 395	Pro	Pro	Val	Leu	Asp 400
Ser	Asp	Gly	Ser	Phe 405	Phe	Leu	Tyr	Ser	Lys 410	Leu	Thr	Val	Asp	Lys 415	Ser
Arg	Trp	Gln	Gln 420	Gly	Asn	Val	Phe	Ser 425	Cys	Ser	Val	Met	His 430	Glu	Ala

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Ser	Gln	Arg	Leu 20	Ser	CAa	Ala	Ala	Ser 25	Gly	Phe	Thr	Phe	Ser 30	Ser	Tyr
Gly	Met	His 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Val
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Cys 145	Leu	Val	Lys	Asp	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Ser	Trp	Asn 160
Ser	Gly	Ala	Leu	Thr 165	Ser	Gly	Val	His	Thr 170	Phe	Pro	Ala	Val	Leu 175	Gln
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Ser	Leu	Gly 195	Thr	Gln	Thr	Tyr	Ile 200	Cys	Asn	Val	Asn	His 205	Lys	Pro	Ser
Asn	Thr 210	Lys	Val	Asp	Lys	Lys 215	Val	Glu	Pro	Lys	Ser 220	CAa	Asp	Lys	Thr
His 225	Thr	Cys	Pro	Pro	Сув 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240
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Ser 305	Val	Leu	Thr	Val	Leu 310	His	Gln	Asp	Trp	Leu 315	Asn	Gly	Lys	Glu	Tyr 320
Lys	Сув	Lys	Val	Ser 325	Asn	Lys	Ala	Leu	Pro 330	Ala	Pro	Ile	Glu	Lys 335	Thr

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Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys 355 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asp Gln Val Ser Asp Gln Val Ser Asp Glu Trp Glu Ser Asp Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp 385 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp 385 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Thr Val Asp Lys Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Asp Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala 420 Arg Trp Gln Gln Gly Asn Val Phe Ser Leu Ser Leu Ser Pro Gly Lys 445	Ile	Ser	Lys	Ala 340	Lys	Gly	Gln	Pro	Arg 345	Glu	Pro	Gln	Val	Tyr 350	Thr	Leu
370	Pro	Pro		Arg	Asp	Glu	Leu		Lys	Asn	Gln	Val		Leu	Trp	Cya
395 396 395 400 395 400 395 400 395 400 395 400 395 400 395 400 395 405 405 405 405 405 405 405 405 405 40	Leu		Tàa	Gly	Phe	Tyr		Ser	Asp	Ile	Ala		Glu	Trp	Glu	Ser
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala 420		Gly	Gln	Pro	Glu		Asn	Tyr	Lys	Thr		Pro	Pro	Val	Leu	_
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys 435 Sep In Companient of the His Asn His Tyr Thr Gln Lys Seq Leu Ser Leu Ser Leu Ser Pro Gly Lys 2210 SEQ ID NO 93 Seq In No 93 Seq In Jens His Lys Pro (2220 FEATURE: Seq In Seq In Jens His In Jen	Ser	Asp	Gly	Ser		Phe	Leu	Tyr	Ser		Leu	Thr	Val	Asp		Ser
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Ser Gln Arg Glu Leu Val Gln Arg Arg Arg Gln Arg Gl	<211 <212 <213 <220	L> LE 2> TY 3> OF 0> FE	ENGTH PE: RGANI EATUR	H: 44 PRT SM: RE:	18 Arti			_		зног	ıE					
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Ala Arg Glu Leu Gly Arg Arg Tyr Phe 105 Asp Leu Trp Gly Arg 110 Thr 110		Gly	Arg	Phe	Thr		Ser	Arg	Asp	Asn		Lys	Asn	Thr	Leu	
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115	Ala	Arg	Glu		Gly	Arg	Arg	Tyr		Asp	Leu	Trp	Gly		Gly	Thr
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Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser 180 Thr 180 Th		Leu	Val	Lys	Asp		Phe	Pro	Glu	Pro		Thr	Val	Ser	Trp	
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	Asn		Lys	Val	Asp	Lys	_	Val	Glu	Pro	Lys		СЛа	Asp	Lys	Thr
		Thr	Cys	Pro	Pro	_	Pro	Ala	Pro	Glu		Leu	Gly	Gly	Pro	

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Lys	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val
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Ala	Val 370	ГЛа	Gly	Phe	Tyr	Pro 375	Ser	Asp	Ile	Ala	Val 380	Glu	Trp	Glu	Ser
Asn 385	Gly	Gln	Pro	Glu	Asn 390	Asn	Tyr	Lys	Thr	Thr 395	Pro	Pro	Val	Leu	Asp 400
Ser	Asp	Gly	Ser	Phe 405	Phe	Leu	Val	Ser	Lys 410	Leu	Thr	Val	Asp	Lys 415	Ser
Arg	Trp	Gln	Gln 420	Gly	Asn	Val	Phe	Ser 425	Cya	Ser	Val	Met	His 430	Glu	Ala
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Asn	Thr 210	Lys	Val	Asp	Lys	Lys 215	Val	Glu	Pro	Lys	Ser 220	Сув	Asp	Lys	Thr
His 225	Thr	Cys	Pro	Pro	Сув 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240
Val	Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp 250	Thr	Leu	Met	Ile	Ser 255	Arg
Thr	Pro	Glu	Val 260	Thr	CÀa	Val	Val	Val 265	Asp	Val	Ser	His	Glu 270	Asp	Pro
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ГÀа	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val
Ser 305	Val	Leu	Thr	Val	Leu 310	His	Gln	Asp	Trp	Leu 315	Asn	Gly	Lys	Glu	Tyr 320
ГÀв	Сув	Lys	Val	Ser 325	Asn	Lys	Ala	Leu	Pro 330	Ala	Pro	Ile	Glu	Lys 335	Thr
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Pro	Pro	Сув 355	Arg	Asp	Glu	Leu	Thr 360	Lys	Asn	Gln	Val	Ser 365	Leu	Trp	Cys
Leu	Val 370	Lys	Gly	Phe	Tyr	Pro 375	Ser	Asp	Ile	Ala	Val 380	Glu	Trp	Glu	Ser
Asn 385	Gly	Gln	Pro	Glu	Asn 390	Asn	Tyr	Lys	Thr	Thr 395	Pro	Pro	Val	Leu	Asp 400
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Arg	Trp	Gln	Gln 420	Gly	Asn	Val	Phe	Ser 425	Сув	Ser	Val	Met	His 430	Glu	Ala
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Gly	Met	His 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Val

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn 145 150 155 160

Ala	Ile 50	Ile	Trp	Phe	Asp	Gly 55	Ser	Ser	Thr	Tyr	Tyr 60	Ala	Asp	Ser	Val
Arg 65	Gly	Arg	Phe	Thr	Ile 70	Ser	Arg	Asp	Asn	Ser 75	Lys	Asn	Thr	Leu	Tyr 80
Leu	Gln	Met	Asn	Ser 85	Leu	Arg	Ala	Glu	Asp 90	Thr	Ala	Val	Tyr	Phe 95	Cys
Ala	Arg	Glu	Leu 100	Gly	Arg	Arg	Tyr	Phe 105	Asp	Leu	Trp	Gly	Arg 110	Gly	Thr
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Leu	Ala 130	Pro	Ser	Ser	Lys	Ser 135	Thr	Ser	Gly	Gly	Thr 140	Ala	Ala	Leu	Gly
Cys 145	Leu	Val	Lys	Asp	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Ser	Trp	Asn 160
Ser	Gly	Ala	Leu	Thr 165	Ser	Gly	Val	His	Thr 170	Phe	Pro	Ala	Val	Leu 175	Gln
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Ser	Leu	Gly 195	Thr	Gln	Thr	Tyr	Ile 200	Cys	Asn	Val	Asn	His 205	Lys	Pro	Ser
Asn	Thr 210	Lys	Val	Asp	Lys	Lys 215	Val	Glu	Pro	Lys	Ser 220	CAa	Asp	Lys	Thr
His 225	Thr	Cys	Pro	Pro	Сув 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240
Val	Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp 250	Thr	Leu	Met	Ala	Ser 255	Arg
Thr	Pro	Glu	Val 260	Thr	Cys	Val	Val	Val 265	Asp	Val	Ser	His	Glu 270	Asp	Pro
Glu	Val	Lys 275	Phe	Asn	Trp	Tyr	Val 280	Asp	Gly	Val	Glu	Val 285	His	Asn	Ala
Lys	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val
Ser 305	Val	Leu	Thr	Val	Leu 310	Ala	Gln	Asp	Trp	Leu 315	Asn	Gly	Lys	Glu	Tyr 320
Lys	Сув	Lys	Val	Ser 325	Asn	Lys	Ala	Leu	Pro 330	Ala	Pro	Ile	Glu	Lys 335	Thr
Ile	Ser	Lys	Ala 340	Lys	Gly	Gln	Pro	Arg 345	Glu	Pro	Gln	Val	Сув 350	Thr	Leu
Pro	Pro	Ser 355	Arg	Aap	Glu	Leu	Thr 360	Lys	Asn	Gln	Val	Ser 365	Leu	Ser	Cys
Ala	Val 370	ГÀа	Gly	Phe	Tyr	Pro 375	Ser	Aap	Ile	Ala	Val 380	Glu	Trp	Glu	Ser
Asn 385	Gly	Gln	Pro	Glu	Asn 390	Asn	Tyr	Lys	Thr	Thr 395	Pro	Pro	Val	Leu	Asp 400
Ser	Asp	Gly	Ser	Phe 405	Phe	Leu	Val	Ser	Lys 410	Leu	Thr	Val	Asp	Lys 415	Ser
Arg	Trp	Gln	Gln 420	Gly	Asn	Val	Phe	Ser 425	Cys	Ser	Val	Met	His 430	Glu	Ala
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Gly	Met	His 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Val
Ala	Ile 50	Ile	Trp	Phe	Asp	Gly 55	Ser	Ser	Thr	Tyr	Tyr 60	Ala	Asp	Ser	Val
Arg 65	Gly	Arg	Phe	Thr	Ile 70	Ser	Arg	Asp	Asn	Ser 75	Lys	Asn	Thr	Leu	Tyr 80
Leu	Gln	Met	Asn	Ser 85	Leu	Arg	Ala	Glu	Asp 90	Thr	Ala	Val	Tyr	Phe 95	CÀa
Ala	Arg	Glu	Leu 100	Gly	Arg	Arg	Tyr	Phe 105	Asp	Leu	Trp	Gly	Arg 110	Gly	Thr
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Cys 145	Leu	Val	Lys	Asp	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Ser	Trp	Asn 160
Ser	Gly	Ala	Leu	Thr 165	Ser	Gly	Val	His	Thr 170	Phe	Pro	Ala	Val	Leu 175	Gln
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Ser 305	Val	Leu	Thr	Val	Leu 310	His	Gln	Asp	Trp	Leu 315	Asn	Gly	Lys	Glu	Tyr 320
ГÀа	Сув	Lys	Val	Ser 325	Asn	Lys	Ala	Leu	Pro 330	Ala	Pro	Ile	Glu	Lys	Thr
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Arg	Trp	Gln	Gln 420	Gly	Asn	Val	Phe	Ser 425	Cys	Ser	Val	Met	His 430	Glu	Ala
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Ala	Arg	Glu	Leu 100	Gly	Arg	Arg	Tyr	Phe 105	Asp	Leu	Trp	Gly	Arg 110	Gly	Thr
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Leu	Ala 130	Pro	Ser	Ser	Lys	Ser 135	Thr	Ser	Gly	Gly	Thr 140	Ala	Ala	Leu	Gly
Cys 145	Leu	Val	Lys	Asp	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Ser	Trp	Asn 160
Ser	Gly	Ala	Leu	Thr 165	Ser	Gly	Val	His	Thr 170	Phe	Pro	Ala	Val	Leu 175	Gln
Ser	Ser	Gly	Leu 180	Tyr	Ser	Leu	Ser	Ser 185	Val	Val	Thr	Val	Pro 190	Ser	Ser
Ser	Leu	Gly 195	Thr	Gln	Thr	Tyr	Ile 200	Сув	Asn	Val	Asn	His 205	Lys	Pro	Ser
Asn	Thr 210	Lys	Val	Asp	ГЛа	Lys 215	Val	Glu	Pro	Lys	Ser 220	СЛа	Asp	Lys	Thr
His 225	Thr	Сув	Pro	Pro	Сув 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240
Val	Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp 250	Thr	Leu	Met	Ile	Ser 255	Arg
Thr	Pro	Glu	Val	Thr	CAa	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro

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ГÀз	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val
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Pro	Pro	Ser 355	Arg	Asp	Glu	Leu	Thr 360	Lys	Asn	Gln	Val	Ser 365	Leu	Ser	Cys
Ala	Val 370	Lys	Gly	Phe	Tyr	Pro 375	Ser	Asp	Ile	Ala	Val 380	Glu	Trp	Glu	Ser
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Ser	Asp	Gly	Ser	Phe 405	Phe	Leu	Val	Ser	Lys 410	Leu	Thr	Val	Asp	Lys 415	Ser
Arg	Trp	Gln	Gln 420	Gly	Asn	Val	Phe	Ser 425	Cys	Ser	Val	Met	His 430	Glu	Ala
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Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Asp 245 250 255	er
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Th His Thr Cys Pro Pro Ala Pro Glu Leu Leu Gly Pro Ser Asp Lys Pro Ser Asp Asp Thr Leu Met Ile Ser Asp Thr Leu Met Ile Ser Asp Asp Thr Leu Met Ile Ser As	er
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys The 210 215 220 220 220 220 220 220 220 220 220 22	
210 215 220 His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Sc 225 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser As 255	er
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Ar 245 250 255	nr
245 250 255	er 10
The Dro Clu Vol The Circ Vol Vol Vol Age Vol Cor Via Clu Age Dr	rg
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pr 260 265 270	ro
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn A 275 280 285	la
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Va 290 295 300	al
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Ty 305 310 315 32	yr 20
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Th 325 330 335	ır
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Le 340 345 350	∍u
Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cy 355 360 365	\a
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Se 370 375 380	∍r
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu As 385 390 395 40	oo OO
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Se 405 410 415	er
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Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr

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Ser	Leu	Gly 195	Thr	Gln	Thr	Tyr	Ile 200	Cys	Asn	Val	Asn	His 205	Lys	Pro	Ser
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				245		-		-	250				Ile	255	
			260		-			265					Glu 270	_	
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	290					295					300		Arg		
305					310					315			Lys		320
				325					330				Glu	335	
			340					345					Сув 350		
		355		Ī			360					365	Leu		
	370					375					380		Trp		
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Ser	Asp	Gly	Ser	Phe 405	Phe	Leu	Val	Ser	Lys 410	Leu	Thr	Val	Asp	Lys 415	Ser
Arg	Trp	Gln	Gln 420	Gly	Asn	Val	Phe	Ser 425	Cys	Ser	Val	Met	His 430	Glu	Ala
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Ala	Arg	Glu	Leu 100	Gly	Arg	Arg	Tyr	Phe 105	Asp	Leu	Trp	Gly	Arg 110	Gly	Thr
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Leu	Ala 130	Pro	Ser	Ser	Lys	Ser 135	Thr	Ser	Gly	Gly	Thr 140	Ala	Ala	Leu	Gly
Cys 145	Leu	Val	Lys	Asp	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Ser	Trp	Asn 160
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Ser	Leu	Gly 195	Thr	Gln	Thr	Tyr	Ile 200	CÀa	Asn	Val	Asn	His 205	Lys	Pro	Ser
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His 225	Thr	Cys	Pro	Pro	Cys 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240
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Thr	Pro	Glu	Val 260	Thr	CAa	Val	Val	Val 265	Asp	Val	Ser	His	Glu 270	Aap	Pro
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rys	Thr 290	ГÀа	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val
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Pro	Pro	Сув 355	Arg	Asp	Glu	Leu	Thr 360	Lys	Asn	Gln	Val	Ser 365	Leu	Trp	Cys
Leu	Val 370	Lys	Gly	Phe	Tyr	Pro 375	Ser	Asp	Ile	Ala	Val 380	Glu	Trp	Glu	Ser

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Ser	Asp	Gly	Ser	Phe 405	Phe	Leu	Tyr	Ser	Lys 410	Leu	Thr	Val	Asp	Lys 415	Ser
Arg	Trp	Gln	Gln 420	Gly	Asn	Val	Phe	Ser 425	Cya	Ser	Val	Met	His 430	Glu	Ala
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Gly	Met	His 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Val
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Asn	Thr 210	Lys	Val	Asp	ГÀа	Lys 215	Val	Glu	Pro	Lys	Ser 220	CAa	Asp	TÀa	Thr
His 225	Thr	Сув	Pro	Pro	Cys 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240
Val	Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp 250	Thr	Asp	Met	Ile	Ser 255	Arg
Thr	Pro	Glu	Val 260	Thr	Cya	Val	Val	Val 265	Asp	Val	Ser	His	Glu 270	Asp	Pro
Glu	Val	Lys 275	Phe	Asn	Trp	Tyr	Val 280	Asp	Gly	Val	Glu	Val 285	His	Asn	Ala

ГÀа	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val	
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Gln	Val	Tyr 355	Thr	Leu	Pro	Pro	Gys	Arg	Asp	Glu	Leu	Thr 365	Lys	Asn	Gln
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Thr	Cys	Pro	Pro	Сув 245	Pro	Ala	Pro	Glu	Leu 250	Leu	Gly	Gly	Pro	Ser 255	Val
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Thr 305	Lys	Pro	Arg	Glu	Glu 310	Gln	Tyr	Asn	Ser	Thr 315	Tyr	Arg	Val	Val	Ser 320	
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Lys Ser	Arg	Trp 420	Gln	Glu	Gly	Asn	Val 425	Phe	Ser	CÀa	Ser	Val 430	Met	His	
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Gly	Asn	Val 435	Phe	Ser	CAa	Ser	Val 440	Met	His	Glu	Ala	Leu 445	Ala	Asn	His
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Ser	His	Glu 275	Asp	Pro	Glu	Val	Lys 280	Phe	Asn	Trp	Tyr	Val 285	Asp	Gly	Val

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Gln '	Val	Cys 355	Thr	Leu	Pro	Pro	Ser 360	Arg	Asp	Glu	Leu	Thr 365	Lys	Asn	Gln
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Thr	Val	Asp	Lys 420	Ser	Arg	Trp	Gln	Gln 425	Gly	Asn	Val	Phe	Ser 430	Cys	Ser
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Leu	Ser 450	Pro	Gly	ГÀв											
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	** 1	th A	AAA n		ions		avy c	chair	120	of <\	/EGF	-ANG-	-2> (AscI	Fab IgG1
<400	> SE	EQUE1	ICE :	nutat 109	cions	3									
Ser	> SE Tyr	EQUEN Val	ICE: Leu	nutat 109 Thr 5	ions Gln	Pro	Pro	Ser	Val 10	Ser	Val	Ala	Pro	Gly 15	Gln
Ser	> SE Tyr Ala	EQUEN Val Arg	NCE: Leu Ile 20	109 Thr 5 Thr	Gln Cys	Pro Gly	Pro	Ser Asn 25	Val 10 Asn	Ser Ile	Val Gly	Ala Ser	Pro Lys 30	Gly 15 Ser	Gln Val
Ser 1 Thr .	> SE Tyr Ala Trp	Val Arg Tyr 35	Leu Ile 20 Gln	109 Thr 5 Thr	Gln Cys Lys	Pro Gly Pro	Pro Gly Gly 40	Ser Asn 25 Gln	Val 10 Asn Ala	Ser Ile Pro	Val Gly Val	Ala Ser Leu 45	Pro Lys 30 Val	Gly 15 Ser Val	Val Tyr
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Ser 1 Thr . His .	> SE Tyr Ala Trp Asp 50	Val Arg Tyr 35 Ser	ICE: Leu Ile 20 Gln Asp	Thr 5 Thr Gln	Gln Cys Lys	Pro Gly Pro Ser 55	Pro Gly Gly 40	Ser Asn 25 Gln	Val 10 Asn Ala Pro	Ser Ile Pro Glu	Val Gly Val Arg	Ala Ser Leu 45 Phe	Pro Lys 30 Val Ser	Gly 15 Ser Val	Gln Val Tyr Ser
Ser 1 Thr . His 4	> SE Tyr Ala Trp Asp 50 Ser	Val Arg Tyr 35 Ser	ICE: Leu Ile 20 Gln Asp	109 Thr 5 Thr Gln Arg	Gln Cys Lys Pro	Pro Gly Pro Ser 55	Pro Gly Gly 40 Gly	Ser Asn 25 Gln Ile	Val 10 Asn Ala Pro	Ser Ile Pro Glu Ser 75	Val Gly Val Arg 60	Ala Ser Leu 45 Phe	Pro Lys 30 Val Ser Glu	Gly 15 Ser Val Gly	Gln Val Tyr Ser Gly 80
Ser 1 Thr . His 4 Asp . Asn .65	> SE Tyr Ala Trp Asp 50 Ser	Val Arg Tyr 35 Ser Gly	Leu Ile 20 Gln Asp Asn	Thr Gln Arg Thr	Gln Cys Lys Pro Ala 70 Tyr	Pro Gly Pro Ser 55 Thr	Pro Gly 40 Gly Leu	Ser Asn 25 Gln Ile Thr	Val 10 Asn Ala Pro Ile Trp 90	Ser Ile Pro Glu Ser 75 Asp	Val Gly Val Arg 60 Arg	Ala Ser Leu 45 Phe Val	Pro Lys 30 Val Ser Glu Ser	Gly Val Ala Asp 95	Gln Val Tyr Ser Gly 80 His
Ser 1 Thr . His 4 Asp . Asn . 65 Asp .	> SE Tyr Ala Trp Asp 50 Ser Glu Val	Val Arg Tyr 35 Ser Gly Ala	ICE: Leu Ile 20 Gln Asp Asn Asp	nutat 109 Thr 5 Thr Gln Arg Thr Tyr 85 Gly	Gln Cys Lys Pro Ala 70 Tyr	Pro Gly Pro Ser 55 Thr Cys	Pro Gly Gly 40 Gly Leu Gln	Ser Asn 25 Gln Ile Thr Val Leu 105	Val 10 Asn Ala Pro Ile Trp 90	Ser Ile Pro Glu Ser 75 Asp	Val Gly Val Arg 60 Arg Leu	Ala Ser Leu 45 Phe Val Ser	Pro Lys 30 Val Ser Glu Ser Gln 110	Gly 15 Ser Val Gly Ala Asp 95 Pro	Gln Val Tyr Ser Gly 80 His
Ser 1 Thr . His 4 Asp . Asn . 65 Asp . Trp 7 Ala .	> SE Tyr Ala Trp 50 Ser Glu Val	Val Arg Tyr 35 Ser Gly Ala Phe	ICE: Leu Ile 20 Gln Asp Asn Asp Ser	Thr Thr Tyr S Gly Val	Gln Cys Lys Pro Ala 70 Tyr Gly	Pro Gly Pro Ser 55 Thr Cys Thr	Pro Gly 40 Gly Leu Gln Lys	Ser Asn 25 Gln Ile Thr Val Leu 105 Pro	Val 10 Asn Ala Pro Ile Trp 90 Thr	Ser Ile Pro Glu Ser 75 Asp Val	Val Gly Val Arg 60 Arg Ser Leu Ser	Ala Ser Leu 45 Phe Val Ser Gly Glu 125	Pro Lys 30 Val Ser Glu Ser Gln 110 Glu	Gly 15 Ser Val Gly Ala Asp 95 Pro	Gln Val Tyr Ser Gly 80 His Lys
Ser 1 Thr . His 4 Asp . Asn . 65 Asp . Trp 7 Ala .	> SE Tyr Ala Trp 50 Ser Glu Val Ala Asn	Val Arg Tyr 35 Ser Gly Ala Phe Pro 115 Lys	ICE: Leu Ile 20 Gln Asp Asn Asp Cly 100 Ser Ala	Thr 5 Thr Gln Arg Thr 55 Thr Uyr 85 Gly Val	Gln Cys Lys Pro Ala 70 Tyr Gly Thr	Pro Gly Pro Ser 55 Thr Cys Thr Leu Val	Pro Gly Gly 40 Gly Leu Gln Lys Phe 120 Cys	Ser Asn 25 Gln Ile Thr Val Leu 105 Pro	Val 10 Asn Ala Pro Ile Trp 90 Thr	Ser Ile Pro Glu Ser 75 Asp Val Ser	Val Gly Val Arg 60 Arg Ser Leu Ser Asp 140	Ala Ser Leu 45 Phe Val Ser Gly Glu 125 Phe	Pro Lys 30 Val Ser Glu Ser Gln 110 Glu Tyr	Gly 15 Ser Val Gly Ala Asp 95 Pro	Gln Val Tyr Ser Gly 80 His Lys Gln Gly
Ser 1 Thr . His 4 Asp . Asn . 65 Asp . Trp . Ala . Ala .	> SE Tyr Ala Trp 50 Ser Glu Val Ala Asn 130 Val	Val Arg Tyr 35 Ser Gly Ala Phe Lys Thr	ICE: Leu Ile 20 Gln Asp Asn Asp Gly 100 Ser Ala	nutat 109 Thr 5 Thr Gln Arg Thr Tyr 85 Gly Val Thr	Gln Cys Lys Pro Ala 70 Tyr Gly Thr Leu Trp 150	Pro Gly Pro Ser 55 Thr Cys Thr Leu Val 135 Lys	Pro Gly 40 Gly Leu Gln Lys Phe 120 Cys	Ser Asn 25 Gln Ile Thr Val Leu 105 Pro Leu Asp	Val 10 Asn Ala Pro Ile Trp 90 Thr	Ser Ile Pro Glu Ser 75 Asp Val Ser Ser Ser	Val Gly Val Arg 60 Arg Ser Leu Ser Asp 140 Pro	Ala Ser Leu 45 Phe Val Ser Gly Glu 125 Phe	Pro Lys 30 Val Ser Glu Ser Gln 110 Glu Tyr	Gly 15 Ser Val Gly Ala Asp 95 Pro Leu Pro	Gln Val Tyr Ser Gly 80 His Lys Gln Gly Gly 160

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Arg	Thr	Pro 515	Glu	Val	Thr	CÀa	Val 520	Val	Val	Asp	Val	Ser 525	His	Glu	Asp
Pro	Glu 530	Val	Lys	Phe	Asn	Trp 535	Tyr	Val	Asp	Gly	Val 540	Glu	Val	His	Asn
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Val	Ser	Val	Leu	Thr 565	Val	Leu	Ala	Gln	Asp 570	Trp	Leu	Asn	Gly	Lys 575	Glu

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Thr	Ile	Ser 595	Lys	Ala	Lys	Gly	Gln 600	Pro	Arg	Glu	Pro	Gln 605	Val	Tyr	Thr
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Asp	Ser	Asp	Gly 660	Ser	Phe	Phe	Leu	Tyr 665	Ser	Lys	Leu	Thr	Val 670	Asp	Lys
Ser	Arg	Trp 675	Gln	Gln	Gly	Asn	Val 680	Phe	Ser	Cys	Ser	Val 685	Met	His	Glu
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Lys	Ser	Arg	Trp 420	Gln	Glu	Gly	Asn	Val 425	Phe	Ser	Cys	Ser	Val 430	Met	His
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Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys 210 $$ 215 $$ 220

_				85					90					95	
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Ala	Ala	Pro 115	Ser	Val	Thr	Leu	Phe 120	Pro	Pro	Ser	Ser	Glu 125	Glu	Leu	Gln
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Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro

_																
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Г	/s	Thr 290	Lys	Pro	Arg	Glu	Glu 295	Gln	Tyr	Asn	Ser	Thr 300	Tyr	Arg	Val	Val
	er 05	Val	Leu	Thr	Val	Leu 310	Ala	Gln	Asp	Trp	Leu 315	Asn	Gly	ГЛа	Glu	Tyr 320
ΓŽ	/s	Сув	Lys	Val	Ser 325	Asn	Lys	Ala	Leu	Pro 330	Ala	Pro	Ile	Glu	Lys 335	Thr
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Le	∍u	Ala	Asn 435	His	Ala	Thr	Gln	Lys 440	Ser	Leu	Ser	Leu	Ser 445	Pro	Gly	

- 1. A polypeptide comprising
- a first polypeptide and a second polypeptide each comprising in N-terminal to C-terminal direction at least a portion of an immunoglobulin hinge region, which comprises one or more cysteine residues, an immunoglobulin CH2-domain and an immunoglobulin CH3-domain,

wherein

- i) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
- ii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or

- iii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S.
- 2. The polypeptide according to claim 1, wherein the polypeptide does not specifically bind to the human FcRn and does specifically bind to Staphylococcal protein A.
- 3. The polypeptide according to claim 1, wherein i) the first polypeptide further comprises the mutations Y349C, T366 S, L368A and Y407V and the second polypeptide comprises the mutations S354C and T366W, or ii) the first polypeptide further comprises the mutations S354C, T366 S, L368A and Y407V and the second polypeptide comprises the mutations Y349C and T366W.

- **4**. The polypeptide according to claim **1**, wherein the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain are of the human IgG1 subclass.
- **5**. The polypeptide according to claim **1**, wherein the first polypeptide and the second polypeptide further comprise the mutations L234A and L235A.
- **6**. The polypeptide according to claim **1**, wherein the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain are of the human IgG2 subclass.
- 7. The polypeptide according to claim 1, wherein the immunoglobulin hinge region, the immunoglobulin CH2-domain and the immunoglobulin CH3-domain are of the human IgG4 subclass.
- **8**. The polypeptide according to claim **1**, wherein the first polypeptide and the second polypeptide further comprise the mutations S228P and L235E.
- **9**. The polypeptide according to claim **1**, wherein the first polypeptide and the second polypeptide further comprise the mutation P329G.
- 10. The polypeptide according to claim 1, wherein the polypeptide is a bispecific antibody.
 - 11. A polypeptide comprising:
 - a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1 and an immunoglobulin CH3-domain of the subclass IgG1,
 - a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1 and an immunoglobulin CH3-domain of the subclass IgG1,
 - a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and a light chain constant domain,
 - a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,
 - wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen,
 - wherein the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a second antigen,
 - wherein i) the first polypeptide comprises the mutations Y349C, T366 S, L368A and Y407V and the second polypeptide comprises the mutations S354C and T366W, or ii) the first polypeptide comprises the mutations S354C, T366 S, L368A and Y407V and the second polypeptide comprises the mutations Y349C and T366W.
 - wherein the first and the second polypeptide further comprise the mutations L234A, L235A and P329G, and

wherein

 the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or

- ii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
- iii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S.

12. A polypeptide comprising:

- a first polypeptide comprising in N-terminal to C-terminal direction a first heavy chain variable domain, an immunoglobulin light chain constant domain, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1 and an immunoglobulin CH3-domain of the subclass IgG1,
- a second polypeptide comprising in N-terminal to C-terminal direction a second heavy chain variable domain, an immunoglobulin CH1-domain of the subclass IgG1, an immunoglobulin hinge region of the subclass IgG1, an immunoglobulin CH2-domain of the subclass IgG1 and an immunoglobulin CH3-domain of the subclass IgG1,
- a third polypeptide comprising in N-terminal to C-terminal direction a first light chain variable domain and an immunoglobulin CH1-domain of the subclass IgG1,
- a fourth polypeptide comprising in N-terminal to C-terminal direction a second light chain variable domain and a light chain constant domain,
- wherein the first heavy chain variable domain and the first light chain variable domain form a first binding site that specifically binds to a first antigen,
- wherein the second heavy chain variable domain and the second light chain variable domain form a second binding site that specifically binds to a second antigen,
- wherein i) the first polypeptide comprises the mutations Y349C, T366 S, L368A and Y407V and the second polypeptide comprises the mutations S354C and T366W, or ii) the first polypeptide comprises the mutations S354C, T366 S, L368A and Y407V and the second polypeptide comprises the mutations Y349C and T366W,
- wherein the first and the second polypeptide further comprise the mutations L234A, L235A and P329G, and

wherein

- the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations H310A, H433A and Y436A, or
- ii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251D, L314D and L432D, or
- iii) the first polypeptide comprises the mutations I253A, H310A and H435A and the second polypeptide comprises the mutations L251S, L314S and L432S
- 13. A polypeptide according to claims 1, 11 or 12 for intravitreal application.
- 14. A polypeptide according to claims 1, 11 or 12 for the treatment of vascular eye diseases.
- 15. A pharmaceutical formulation comprising a polypeptide according to claims 1, 11 or 12 and optionally a pharmaceutically acceptable carrier.
 - 16.-18. (canceled)
- 19. A method for the transport of a soluble receptor ligand from the eye over the blood-ocular-barrier into the blood

circulation in an individual comprising administering to the individual an effective amount of the polypeptide according to claim 1, 11 or 12.

- 20. A method for the removal of one or more soluble receptor ligands from the eye in an individual comprising administering to the individual an effective amount of the polypeptide according to claim 1, 11 or 12.

 21. A method for treatment of an eye disease with the
- polypeptide according to claim 1, 11 or 12.