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

(19) World Intellectual Property

Organization

International Bureau



(43) International Publication Date 8 October 2015 (08.10.2015)

- (51) International Patent Classification: *C07K 16/28* (2006.01) *C07K 16/24* (2006.01) *C07K 16/22* (2006.01)
- (21) International Application Number: PCT/EP2015/057165
- (22) International Filing Date:
- 1 April 2015 (01.04.2015)
- (25) Filing Language:English(26) Publication Language:English
- (30) Priority Data: 14163165.5 2 April 2014 (02.04.2014) EP 14179034.5 30 July 2014 (30.07.2014) EP
- (71) Applicant (for all designated States except US): F. HOFF-MANN-LA ROCHE AG [CH/CH]; Grenzacher Strasse 124, CH-4070 Basel (CH).
- (71) Applicant (for US only): HOFFMANN-LA ROCHE INC. [US/US]; Great Notch, 150 Clove Road, 8th Floor, Little Falls, New Jersey 07424 (US).
- (72) Inventors: SCHAEFER, Wolfgang; Tannhaeuserring 190, 68199 Mannheim (DE). KLEIN, Christian; Chruezacherweg 41, CH-8906 Bonstetten (CH). IMHOF-JUNG, Sabine; Egenhofenstr. 26 b, 82152 Planegg (DE). KLOSTERMANN, Stefan; Kernbauernstr. 8, 82061 Neuried (DE). MOLHOJ, Michael; Fachnerstr. 7A, 80686 Muenchen (DE). REGULA, Joerg Thomas; Innerkoflerstr. 17 B, 81377 Muenchen (DE).

(10) International Publication Number WO 2015/150447 A1

- (74) Agents: MERTES, Maria et al.; Roche Diagnostics GmbH, Patent Department (LPP.....6164), P.O.Box 11 52, 82372 Penzberg (DE).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

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

WO 2015/150447 A1

(54) Title: MULTISPECIFIC ANTIBODIES

(57) Abstract: The present invention relates to multispecific antibodies, their manufacture and use.

Multispecific antibodies

The present invention relates to novel multispecific antibodies, their manufacture and use.

Background of the Invention

Engineered proteins, such as bi- or multispecific antibodies capable of binding two or more antigens are known in the art. Such multispecific binding proteins can be generated using cell fusion, chemical conjugation, or recombinant DNA techniques.

A wide variety of recombinant multispecific antibody formats have been developed in the recent past, e.g. tetravalent bispecific antibodies by fusion of, e.g. an IgG antibody format and single chain domains (see e.g. Coloma, M.J., et. al., Nature Biotech. 15 (1997) 159-163; WO 2001/077342; and Morrison, S.L., Nature Biotech. 25 (2007) 1233-1234).

Also several other new formats wherein the antibody core structure (IgA, IgD, IgE, IgG or IgM) is no longer retained such as dia-, tria- or tetrabodies, minibodies, several single chain formats (scFv, Bis-scFv), which are capable of binding two or more antigens, have been developed (Holliger, P., et. al, Nature Biotech. 23 (2005) 1126-1136; Fischer, N., and Léger, O., Pathobiology 74 (2007) 3-14; Shen, J., et. al., J. Immunol. Methods 318 (2007) 65-74; Wu, C., et al., Nature Biotech. 25 (2007) 1290-1297).

All such formats use linkers either to fuse the antibody core (IgA, IgD, IgE, IgG or IgM) to a further binding protein (e.g. scFv) or to fuse e.g. two Fab fragments or scFv (Fischer, N., and Léger, O., Pathobiology 74 (2007) 3-14). While it is obvious that linkers have advantages for the engineering of bispecific antibodies, they may also cause problems in therapeutic settings. Indeed, these foreign peptides might elicit an immune response against the linker itself or the junction between the protein and the linker. Furthermore, the flexible nature of these peptides makes them more prone to proteolytic cleavage, potentially leading to poor antibody stability, aggregation and increased immunogenicity. In addition one may want to retain effector functions, such as e.g. complement-dependent cytotoxicity (CDC) or antibody dependent cellular cytotoxicity (ADCC), which are mediated through the Fc-part by maintaining a high degree of similarity to naturally occurring antibodies.

10

5

25

Thus, ideally, one should aim at developing bispecific antibodies that are very similar in general structure to naturally occurring antibodies (like IgA, IgD, IgE, IgG or IgM) with minimal deviation from human sequences.

In one approach bispecific antibodies that are very similar to natural antibodies 5 have been produced using the quadroma technology (see Milstein, C., and Cuello, A.C., Nature 305 (1983) 537-540) based on the somatic fusion of two different hybridoma cell lines expressing murine monoclonal antibodies with the desired specificities of the bispecific antibody. Because of the random pairing of two different antibody heavy and light chains within the resulting hybrid-hybridoma (or 10 quadroma) cell line, up to ten different antibody species are generated of which only one is the desired, functional bispecific antibody. Due to the presence of mispaired byproducts, and significantly reduced production yields, sophisticated purification procedures are required (see e.g. Morrison, S.L., Nature Biotech. 25 (2007) 1233-1234). In general the same problem of mispaired by-products remains 15 if recombinant expression techniques are used.

An approach to circumvent the problem of mispaired byproducts, which is known as 'knobs-into-holes', aims at forcing the pairing of two different antibody heavy chains by introducing mutations into the CH3 domains to modify the contact interface. On one chain bulky amino acids were replaced by amino acids with short 20 side chains to create a 'hole'. Conversely, amino acids with large side chains were introduced into the other CH3 domain, to create a 'knob'. By coexpressing these two heavy chains (and two identical light chains, which have to be appropriate for both heavy chains), high yields of heterodimer formation ('knob-hole') versus homodimer formation ('hole-hole' or 'knob-knob') was observed (Ridgway, J.B., et al., Protein Eng. 9 (1996) 617-621; and WO 96/027011). The percentage of heterodimer could be further increased by remodeling the interaction surfaces of the two CH3 domains using a phage display approach and the introduction of a disulfide bridge to stabilize the heterodimers (Merchant, A.M., et al., Nature Biotech. 16 (1998) 677-681; Atwell, S., et al., J. Mol. Biol. 270 (1997) 26-35). 30 New approaches for the knobs-into-holes technology are described in e.g. in EP 1 870 459 A1. Although this format appears very attractive, no data describing progression towards the clinic are currently available. One important constraint of this strategy is that the light chains of the two parent antibodies have to be identical to prevent mispairing and formation of inactive molecules. Thus this technique is

not appropriate as a basis for easily developing recombinant, tri-or tetraspecific antibodies against three or four antigens starting from two antibodies against the first and the second antigen, as either the heavy chains of these antibodies and/or the identical light chains have to be optimized first and then further antigen binding peptides against the third and fourth antigen have to be added.

WO 2006/093794 relates to heterodimeric protein binding compositions. WO 99/37791 describes multipurpose antibody derivatives. Morrison, S.L., et al., J. Immunol. 160 (1998) 2802-2808 refers to the influence of variable region domain exchange on the functional properties of IgG.

- 10 WO 2013/02362 relates to heterodimerized polypeptides. WO 2013/12733 relates to polypeptides comprising heterodimeric Fc regions. WO 2012/131555 relates to engineered hetero-dimeric immunoglobulins. EP 2647707 relates to engineered hetero-dimeric immunoglobulins.
- WO 2013/026835 relates to bispecific, Fc free antibodies with a domain crossover.
 WO 2009/080251, WO 2009/080252, WO 2009/080253, WO 2009/080254 and Schaefer, W. et al, PNAS, 108 (2011) 11187-1191 relate to bivalent, bispecific IgG antibodies with a domain crossover.
- The multispecific antibodies with VH/VL replacement/exchange in one binding to prevent light chain mispairing (CrossMab^{VH-VL}) which are described in WO2009/080252, (see also Schaefer, W. et al, PNAS, 108 (2011) 11187-1191) clearly reduce the byproducts caused by the mismatch of a light chain against a first antigen with the wrong heavy chain against the second antigen (compared to approaches without such domain exchange). However their preparation is not completely free of side products. The main side product is based on a Bence–Jones -type interaction -see also Schaefer, W. et al, PNAS, 108 (2011) 11187-1191; in Fig. S1I of the Supplement).

Therefore there is still a need for further reduction of such side products improve e.g. the yield of such bispecific antibodies.

Summary of the Invention

30 The invention relates to a multispecific antibody, comprising:

5

10

15

20

- 4 -

- a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and
- b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

wherein

- i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index); or
 - ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

A further embodiment of the invention is a method for the preparation of a multispecific antibody according to the invention

comprising the steps of

A) transforming a host cell with vectors comprising nucleic acid molecules encoding

a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and

25

b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

5 wherein

- i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index); or
- ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).
 - B) culturing the host cell under conditions that allow synthesis of said antibody molecule; and
 - C) recovering said antibody molecule from said culture.
- A further embodiment of the invention is a nucleic acid encoding the amino acid sequences of a multispecific antibody according to the invention.
 - A further embodiment of the invention are expression vectors containing the nucleic acid according to the invention capable of expressing said nucleic acid in a host cell.

25

10

15

- A further embodiment of the invention is a host cell comprising a vector according to the invention.
- A further embodiment of the invention is a composition, e.g. a pharmaceutical or a diagnostic composition, of the antibody according to the invention.
- 5 A further embodiment of the invention is a pharmaceutical composition comprising an antibody according to the invention and at least one pharmaceutically acceptable excipient.
 - A further embodiment of the invention is a method for the treatment of a patient in need of therapy, characterized by administering to the patient a therapeutically effective amount of an antibody according to the invention.
 - According to the invention, the ratio of a desired multispecific antibody compared to undesired main side Bence Jones-type products can be improved by the introduction of substitutions of charged amino acids with the opposite charges at specific amino acid positions in the CH1 and CL domains.

15 **Description of the Figures**

10

20

25

Figure 1	Some examples of multispecific antibodies according to the
	invention with VH/VL domain replacement in one antibody
	binding arm and specific mutations in one CH1/CL domain
	interface:
	at least the amino acid at position 124 of the CL domain is
	substituted independently by lysine (K), arginine (R) or Histidine
	(H) (numbering according to Kabat), and
	at least the amino acid at position 147 of the CH1 domain or the
	amino acid at position 213 of the CH1 domain is substituted
	independently by glutamic acid (E), or aspartic acid (D)
	(numbering according to Kabat EU index).
	Figure 1A: VH/VL domain replacement in one antibody binding
	arm and specific mutations in the CH1/CL domain interface of

the other antibody binding arm.

Figure 1B: VH/VL domain replacement in one antibody binding arm and specific mutations in the CH1/CL domain interface of the same antibody binding arm. Figure 1C: VH/VL domain replacement in one antibody binding 5 arm and specific mutations in the CH1/CL domain interface of the other antibody binding arm, and modifications of the CH3/CH3 domain interface to enforce heavy chain heterodimerization (like e.g. knobs-into-holes technology or alternative heterodimerization technologies like e.g. substitution 10 of charged amino acids with their respective opposite charge). **Figure 2A** Example of multispecific antibody with VH/VL domain replacement in one antibody binding arm and without mutations in one CH1/CL domain interface (left side) and the main side 15 product of this multispecific antibody (due to VL-VL Bence jones-type domain interaction)- other possible variants as potential side products were not detected neither by mass spectrometry directly; nor by mass spectrometry after plasmin or LysC digestion by analyzing the Fab fragments thereof. 20 **Figure 2B** Origin of the main side product of multispecific antibody with VH/VL domain replacement in one antibody binding arm and without mutations in one CH1/CL domain interface (due to VL-VL Bence jones-type domain interaction). 25 **Figure 3** Figure 3A: wild type (wt) amino acid sequences in CH1 domain (two IgG isotypes are shown) with underlined and highlighted amino acid positions 147 and 213 (numbering according to Kabat EU index). Figure 3B: wild type (wt) amino acid sequences in the CL 30 domain of kappa isotype with underlined and highlighted amino acid positions 124 and 123 (numbering according to Kabat). Figure 3C: wild type (wt) amino acid sequences in the CL domain of lambda isotype with underlined and highlighted amino acid positions 124 and 123 (numbering according to Kabat). 35

- 7 -

	Figure 4	Figure 4A: Reduction of main Bence-Jones-type side product by single charged amino acids substitutions according to the invention in the CH1/CL interface.
5		Examples of anti- <i>Ang2-VEGF</i> multispecific antibodies according to the invention with VH/VL domain exchange/replacement (<i>CrossMAb</i> ^{Vh-VL}).
		Comparison of wild type (wt) and different combinations of single charged amino acids substitutions
10		1) wildtype (wt) anti- <i>Ang2-VEGF CrossMAb</i> ^{Vh-VL} multispecific antibody without specific amino acid substitutions in the CH1/CL interface,
		2) anti- <i>Ang2-VEGF</i> multispecific antibodies according to the invention i) with substitutions at position 124 of the CL domain is, and at position 147 of the CH1 domain (numbering according
15		to Kabat EU index) or ii) with substitutions at position 124 of the CL domain is, and at position 213 of the CH1 domain (numbering according to Kabat EU index),
		3) other anti- <i>Ang2-VEGF CrossMAb</i> ^{Vh-VL} multispecific antibodies with substitutions at different positions
20		Figure 4B: Sequences (SEQ ID NOs) of the multispecific antibodies for which the results are shown in Figure 4A.
	Figure 5	Figure 5A: Reduction of main Bence-Jones-type side product by different charged amino acids substitutions in the CH1/CL
25		interface. Examples of anti- <i>Ang2-VEGF</i> multispecific antibodies according to the invention with VH/VL domain exchange/replacement (<i>CrossMAb</i> ^{Vh-VL}).
30		Comparison of wild type (wt) and different combinations of charged amino acids substitutions
		Figure 5B: Sequences (SEQ ID NOs) of the multispecific antibodies for which the results are shown in Figure 5A.
	Figure 6	Figure 6A:Reduction of main Bence-Jones-type product side by
35		different charged amino acids substitutions in the CH1/CL

-9-

• • •	
interface	
micrace	٠

5		 Examples of anti-<i>IL-17/TWEAK</i> multispecific antibodies according to the invention with VH/VL domain exchange/replacement (<i>CrossMAb</i>^{Vh-VL}). Comparison of wild type (wt) and different combinations of charged amino acids substitutions Figure 6B: Sequences (SEQ ID NOs) of the multispecific antibodies for which the results are shown in Figure 6A.
10	Figure 7	Some examples of bivalent multispecific antibodies according to the invention with VH/VL domain replacement in one antibody binding arm and specific mutations in one CH1/CL domain interface, wherein the multispecific antibdodies are devoid of an Fc fragment (Fab-CrossFab ^{VH-VL} format and CrossFab ^{VH-VL} -Fab):
15		at least the amino acid at position 124 of the CL domain is substituted independently by lysine (K), arginine (R) or Histidine (H) (numbering according to Kabat), and at least the amino acid at position 147 of the CH1 domain or the amino acid at position 213 of the CH1 domain is substituted
20		 independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index). Figure 7A: VH/VL domain replacement in one antibody binding arm and specific mutations in the CH1/CL domain interface of the other antibody binding arm.
25		 Figure 7B: VH/VL domain replacement in one antibody binding arm and specific mutations in the CH1/CL domain interface of the same antibody binding arm. Figure 7C: VH/VL domain replacement in one antibody binding arm with specific mutations in the CH1/CL domain interface of
30		the same antibody binding arm; and further specific mutations in the CH1/CL domain interface of the other antibody binding arm. Figure 7D: VH/VL domain replacement in one antibody binding arm and specific mutations in the CH1/CL domain interface of the other antibody binding arm.
35		-

	Figure 8	Some examples of trivalent multispecific antibodies according to the invention with VH/VL domain replacement in one antibody binding arm and specific mutations in one CH1/CL domain interface, wherein the multispecific antibdodies are devoid of an
5		Fc fragment (Fab-Fab-CrossFab ^{VH-VL} format):
		at least the amino acid at position 124 of the CL domain is substituted independently by lysine (K), arginine (R) or Histidine
		(H) (numbering according to Kabat), and
10		at least the amino acid at position 147 of the CH1 domain or the
10		amino acid at position 213 of the CH1 domain is substituted
		independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).
		Figure 8A, B, C: VH/VL domain replacement in one antibody
		binding arm and specific mutations in the CH1/CL domain
15		interface of the other antibody binding arms.
		Figure 8D: VH/VL domain replacement in one antibody binding
		arm and specific mutations in the CH1/CL domain interface of the same antibody binding arm.
		Figure 8E: VH/VL domain replacement in one antibody binding
20		arm with specific mutations in the CH1/CL domain interface of
		the same antibody binding arm; and further specific mutations in
		the CH1/CL domain interface of the other antibody binding arms.
	Figure 9	Some examples of tetravalent multispecific antibodies according
25		to the invention with VH/VL domain replacement in one
		antibody binding arm and specific mutations in one CH1/CL
		domain interface, wherein the multispecific antibdodies are
		devoid of an Fc fragment (Fab-Fab-CrossFab ^{VH-VL} format):
		at least the amino acid at position 124 of the CL domain is
30		substituted independently by lysine (K), arginine (R) or Histidine
		(H) (numbering according to Kabat), and
		at least the amino acid at position 147 of the CH1 domain or the
		amino acid at position 213 of the CH1 domain is substituted

independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

Figure 9A: VH/VL domain replacement in one antibody binding arm and specific mutations in the CH1/CL domain interface of the other antibody binding arms.

Figure 9B: VH/VL domain replacement in one antibody binding arm and specific mutations in the CH1/CL domain interface of the same antibody binding arm.

10 Detailed Description of the Invention

Multispecific antibodies with a domain replacement/exchange in one binding arm (CrossMabVH-VL) are described in detail in WO2009/080252 and Schaefer, W. et al, PNAS, 108 (2011) 11187-1191 (which are incorporated as reference herein). They clearly reduce the byproducts caused by the mismatch of a light chain against a first antigen with the wrong heavy chain against the second antigen (compared to approaches without such domain exchange). However their preparation is not completely free of side products. The main side product is based on a Bence–Jones –type interaction -see also Schaefer, W. et al, PNAS, 108 (2011) 11187-1191; in Fig. S1I of the Supplement).

20 Therefore we have found now a an approach for further reduction of such side products to improve the yield of such multispecific antibodies (i.e. multispecific antibodies, which comprise a VH/VL domain replacement/exchange only in the binding arm(s) of one antigen specificity, whereas the binding arm(s) of the other antigen specificity does not comprise a VH/VL domain replacement/exchange but rather is of a wild-type antibody domain arrangement as indicated in Fig. 1) by the introduction of substitutions of charged amino acids with the opposite charge at specific amino acid positions in the CH1 and CL domains.

Therefore the invention relates to a multispecific antibody, comprising:

a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and

5

15

 b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

5 wherein

10

15

20

25

- i) in the constant domain CL of the first light chain under a) the amino acid at position 124 (numbering according to Kabat) is substituted by a positively charged amino acid, and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 (numbering according to Kabat EU index) is substituted by a negatively charged amino acid; or
- ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 (numbering according to Kabat) is substituted by a positively charged amino acid, and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at position 147 or the amino acid at position 213 (numbering according to Kabat EU index) is substituted by a negatively charged amino acid.

In accordance with the concept of the invention, the antibody according to the invention comprises only one of the modifications as indicated under i) and ii) above and below. Hence, the multispecific antibody according to the invention comprises either

 in the constant domain CL of the first light chain under a) a substitution of the amino acid at position 124 (numbering according to Kabat) by a positively charged amino acid, and in the constant domain CH1 of the first heavy chain under a) a substitution of the amino acid at position 147 or the amino acid at position 213 (numbering according to Kabat EU index) by a negatively charged amino acid;

or

30

ii) in the constant domain CL of the second light chain under b) a substitution of the amino acid at position 124 (numbering according to Kabat) by a positively charged amino acid, and in the constant domain CH1 of the second heavy chain under b) a substitution of the amino acid at position 147 or the amino acid at position 213 (numbering according to Kabat EU index) by a negatively charged amino acid,

with the proviso that the multispecific antibody does not comprise both modifications mentioned under i) and ii).

Therefore the invention relates to a multispecific antibody, comprising:

- a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and
- b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

wherein

- i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index); or
 - ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at positions 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

5

10

15

20

The invention further relates to a multispecific antibody, comprising:

- a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and
- b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and
- wherein
- i) in the constant domain CL of the first light chain under a) the amino acid at
 position 124 (numbering according to Kabat) is substituted by a positively
 charged amino acid, and wherein in the constant domain CH1 of the first
 heavy chain under a) the amino acid at position 147 or the amino acid at
 position 213 (numbering according to Kabat EU index) is substituted by a

15

20

25

5

The invention further relates to a multispecific antibody, comprising:

- a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and
- b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

wherein

i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted

independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

Thus for said second antibody which specifically binds to a second antigen comprised in a multispecific antibody according to the invention the following applies:

- within the light chain the variable light chain domain VL is replaced by the variable heavy chain domain VH of said antibody; and
- within the heavy chain the variable heavy chain domain VH is replaced by the variable light chain domain VL of said antibody; and
- the constant domains CL and CH1 in the second light chain and second heavy chain of the second antibody are <u>not</u> replaced by each other (remain unexchanged).

Thus for said antibody which specifically binds to a first antigen comprised in a multispecific antibody according to the invention the following applies:

- within said first light chain derived from said first antibody the sequential arrangement of the domains of the light chain (CL-VL) remains unaltered; and
- within said first heavy chain derived from said first antibody the sequential arrangement of the domains of the heavy chain (e.g. CH1-VH or CH3-CH2-CH1-VH) remains unaltered (therefore said antibody which specifically binds to the first antibody does not include a domain exchange, particularly not an exchange of VH/VL).

In other words, said antibody which specifically binds to a first antigen comprised in a multispecific antibody according to the invention comprises:

- a first light chain derived from said first antibody comprising a sequential arrangement of the domains of the light chain of VL-CL (from N-terminal to C-terminal direction); and
 - a first heavy chain derived from said first antibody comprising a sequential arrangement of the domains of the heavy chain of CH1-VH (from from N-terminal to C-terminal direction) (in one embodiment the first heavy chain comprises a sequential arrangement of the domains of the heavy chain of CH3-CH2-CH1-VH from N-terminal to C-terminal direction).

10

15

20

30

- 16 -

The "light chain of an antibody" as used herein is a polypeptide comprising in N-terminal to C-terminal direction an antibody light chain variable domain (VL), and an antibody light chain constant domain (CL), abbreviated as VL-CL.

The "heavy chain of an antibody" as used herein is a polypeptide comprising in Nterminal to C-terminal direction an antibody heavy chain variable domain (VH) and an antibody constant heavy chain domain 1 (CH1).

In one embodiment of the invention the heavy chain of the multispecific antibody includes in N-terminal to C-terminal direction an antibody heavy chain variable domain (VH) and an antibody constant heavy chain domain 1 (CH1) and is devoid of heavy chain constant domains CH2 and CH3, thus abbreviated as VH-CH1. In one embodiment multispecific antibodies according to the invention comprise at least two Fab fragments, wherein the first Fab fragment comprises at least one antigen binding site specific for a first antigen; and the second Fab fragment comprises at least one antigen binding site specific for a second antigen, wherein in the second Fab fragment the variable domains VL and VH in the second light chain and second heavy chain are replaced by each other; and wherein the multispecific antibody is devoid of an Fc domain; and wherein

- i) in the constant domain CL of the light chain of the first Fab fragment the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the heavy chain of the first Fab fragment the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index); or
- ii) in the constant domain CL of the light chain of the second Fab fragment the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or Histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the heavy chain of the second Fab fragment the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

10

5

15

20

25

WO 2015/150447

- 17 -

In a further embodiment multispecific antibodies according to the invention comprise at least two Fab fragments, wherein the first Fab fragment comprises at least one antigen binding site specific for a first antigen; and the second Fab fragment comprises at least one antigen binding site specific for a second antigen, wherein in the second Fab fragment the variable domains VL and VH in the second light chain and second heavy chain are replaced by each other; and wherein the multispecific antibody is devoid of an Fc domain; and wherein

i) in the constant domain CL of the light chain of the first Fab fragment the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the heavy chain of the first Fab fragment the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

As used herein, "Fab fragment" refers to an antibody fragment comprising a light chain fragment comprising a variable VL domain and a constant domain of a light chain (CL), and a variable VH domain and a first constant domain (CH1) of a heavy chain. The multispecific antibodies according to this embodiment comprise 20 at least two Fab fragments, wherein the variable regions of the heavy and light chain of the second Fab fragment are exchanged. Due to the exchange of the variable regions, said second Fab fragment is also referred to as "cross-Fab fragment" or "xFab fragment" or "crossover Fab fragment". In said second Fab fragment wherein the variable regions of the Fab heavy and light chain are 25 exchanged, the crossover Fab molecule comprises a modified heavy chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1), and a modified light chain composed of the heavy chain variable region (VH) and the light chain constant region (CL). This crossover Fab molecule is also referred to as CrossFab^{VH/VL}.

30 The term "Fc domain" is used herein to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. For example in natural antibodies, the Fc domain is composed of two identical protein fragments, derived from the second and third constant domains of the

5

10

5

10

15

20

25

antibody's two heavy chains in IgG, IgA and IgD isotypes; IgM and IgE Fc domains contain three heavy chain constant domains (CH domains 2–4) in each polypeptide chain. "Devoid of the Fc domain" as used herein means that the bispecific antibodies of the invention do not comprise a CH2, CH3 and CH4 domain; i.e. the constant heavy chain consists solely of one or more CH1 domains.

In one embodiment the first and second Fab fragments are connected via a peptide linker. The term "peptide linker" as used herein denotes a peptide with amino acid sequences, which is preferably of synthetic origin. In one embodiment a peptide linker is used to connect one of the Fab fragments to the C- or N-terminus of the other Fab fragment in order to form a multispecific antibody according to the invention. In one preferred embodiment said peptide linker is a peptide with an amino acid sequence with a length of at least 5 amino acids, in one embodiment with a length of 5 to 100, in a further embodiment of 10 to 50 amino acids. In one embodiment said peptide linker is $(GxS)_n$ or $(GxS)_nG_m$ with G = glycine, S =serine, and (x = 3, n = 3, 4, 5 or 6, and m = 0, 1, 2 or 3) or (x=4, n=2, 3, 4 or 5 and m= 0, 1, 2 or 3), in one embodiment said peptide linker is $(G_4S)_2$. The peptide linker is used to connect the first and the second Fab fragment. In one embodiment the first Fab fragment is connected to the C- or N- terminus of the second Fab fragment.

In another preferred embodiment of the invention the heavy chain of an antibody comprises in N-terminal to C-terminal direction an antibody heavy chain variable domain (VH), an antibody constant heavy chain domain 1 (CH1), an antibody heavy chain constant domain 2 (CH2), and an antibody heavy chain constant domain 3 (CH3), abbreviated as VH-CH1-CH2-CH3.

In case the multispecific antibody comprises the domains VH-CH1-CH2-CH3 in each heavy chain, an additional aspect of the invention is to further improve the ratio of a desired multispecific antibody compared to undesired side products can be by modifications of the first and second CH3 domain of said the multispecific antibody to increase the heterodimerisation of both heavy chains containing these first and second CH3 domain.

30

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 homdimers 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 and Bence-Jones type side products.

In one 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 are altered to support heterodimerization by

- substituting at least one amino acid of the CH3 domain of the first heavy chain, and
- substituting at least one amino acid of the CH3 domain of the second heavy chain, wherein said amino acid is facing the at least one amino acid of the CH3 domain of the first heavy chain within the tertiary structure of the multispecific antibody,
- 25 wherein the respective amino acids within the CH3 domains of the first and second heavy chain, respectively, are either
 - substituted such that amino acids of opposite side chain charges are introduced into the opposing heavy chains, or
 - substituted such that amino acids with large and small side chain volumes are introduced into the opposing heavy chains, whereby a protuberance is created by an amino acid with a large side chain volume in one CH3 domain, which is positionable in a cavity located within the other CH3

5

10

15

20

20

25

30

domain, wherein the cavity is created by an amino acid with a small side chain volume.

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 are altered by the "knob-intohole" 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; and WO 98/ 050431. In this method the interaction surfaces of the two CH3 domains are altered to increase the heterodimerisation of both heavy chains containing these two CH3 domains. Each of the two CH3 domains (of the two heavy chains) can be the "knob", while the other is the "hole". The introduction of a disulfide bridge 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.

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

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,

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

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

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

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

and

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

so that within the original interface of the CH3 domain of the other heavy chain that meets the original interface of the CH3 domain of the one heavy chain within the multispecific antibody an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the interface of the CH3 domain of the other heavy chain within which a protuberance within the interface of the CH3 domain of the one heavy chain is positionable.

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

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

In one aspect of the invention both CH3 domains are further altered by the introduction of cysteine (C) as amino acid in the corresponding positions of each CH3 domain such that a disulfide bridge between both CH3 domains can be formed. Thus according to this aspect of the invention, the CH3 domain of the one heavy chain is further altered so that within the original interface of the CH3 domain of the one heavy chain within the multispecific antibody, an amino acid residue is replaced by a cysteine (C) residue, and the CH3 domain of the other heavy chain is further altered so that within the original interface of the other heavy chain is further altered so that within the original interface of the other heavy chain is further altered so that within the original interface of the CH3 domain of the other heavy chain that meets the original interface of the CH3 domain of the other heavy chain that meets the original interface of the CH3 domain of the other heavy chain that meets the original interface of the CH3 domain of the other heavy chain that meets the original interface of the CH3 domain of the other heavy chain that meets the original interface of the CH3 domain of the other heavy chain that meets the original interface of the CH3 domain of the other heavy chain that meets the original interface of the CH3 domain of the one heavy chain within the multispecific antibody, an amino acid residue is replaced by a cysteine (C) residue, such that a disulfide bridge between both CH3 domains can be formed via the introduced cysteine residues.

30 In one preferred embodiment, said multispecific antibody comprises an amino acid T366W mutation in one CH3 domain of the "knob chain" and amino acid T366S, L368A, Y407V mutations in the other CH3 domain of the "hole chain". An additional interchain disulfide bridge between the CH3 domains can also be used

10

15

(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".

In one preferred embodiment, said multispecific antibody (which comprises a CH3 domain in each heavy chain) comprises amino acid S354C and T366W mutations in one CH3 domain and amino acid Y349C, T366S, L368A and Y407V mutations in the other of the two CH3 domains (with 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) (numberings according to Kabat EU index).

 Other techniques for CH3-modifications to enforce 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 and WO 2013/096291.

In one embodiment the heterodimerization approach described in EP 1 870 459A1 is used alternatively. This approach is based on 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 antibodies are amino acid R409D and K370E mutations in the CH3 domain of one heavy chain and amino acid D399K and E357K mutations in the CH3 domain of the other heavy chain of the multispecific antibody (numberings according to Kabat EU index).

In another embodiment said multispecific antibody comprises an amino acid T366W mutation in the CH3 domain of the "knobs chain" and amino acid T366S, L368A and Y407V mutations in the CH3 domain of the "hole chain"; and additionally comprises amino acid R409D and K370E mutations in the CH3 domain of the "knobs chain" and amino acid D399K and E357K mutations in the 30 CH3 domain of the "hole chain".

In another embodiment said multispecific antibody comprises amino acid S354C and T366W mutations in of the CH3 domain of one heavy chain and amino acid

15

Y349C, T366S, L368A and Y407V mutations in the CH3 domain of the other heavy chain; or said multispecific antibody comprises amino acid Y349C and T366W mutations in the CH3 domain of one heavy chain and amino acid S354C, T366S, L368A and Y407V mutations in the CH3 domain of the other heavy chain and additionally comprises amino acid R409D and K370E mutations in the CH3 domain of the "knobs chain" and amino acid D399K and E357K mutations in the CH3 domain of the "hole chain".

In one embodiment the heterodimerization approach described in WO2013/157953 is used alternatively. In one embodiment the CH3 domain of one heavy chain comprises an amino acid T366K mutation and the CH3 domain of the other heavy chain comprises an amino acid L351D mutation. In a further embodiment the CH3 domain of the one heavy chain further comprises an amino acid L351K mutation. In a further embodiment the CH3 domain of the other heavy chain further comprises an amino acid mutation selected from Y349E, Y349D and L368E (in one embodiment L368E).

In one embodiment the heterodimerization approach described in WO2012/058768 is used alternatively. In one embodiment the CH3 domain of one heavy chain comprises amino acid L351Y and Y407A mutations and the CH3 domain of the other heavy chain comprises amino acid T366A and K409F mutations. In a further embodiment the CH3 domain of the other heavy chain further comprises an amino acid mutation at position T411, D399, S400, F405, N390 or K392. In one embodiment said amino acid mutation is selected from the group consisting of

a) T411N, T411R, T411Q, T411K, T411D, T411E and T411W,

b) D399R, D399W, D399Y and D399K,

c) S400E, S400D, S400R and S400K,

d) F405I, F405M, F405T, F405S, F405V and F405W,

e) N390R, N390K and N390D,

f) K392V, K392M, K392R, K392L, K392F and K392E.

10

15

20

In a further embodiment the CH3 domain of one heavy chain comprises amino acid L351Y and Y407A mutations and the CH3 domain of the other heavy chain comprises amino acid T366V and K409F mutations. In a further embodiment the CH3 domain of one heavy chain comprises an amino acid Y407A mutation and the CH3 domain of the other heavy chain comprises amino acid T366A and K409F mutations. In a further embodiment the CH3 domain of the other heavy chain comprises amino acid T366A and K409F mutations. In a further embodiment the CH3 domain of the other heavy chain comprises amino acid T366A and K409F mutations. In a further embodiment the CH3 domain of the other heavy chain further comprises amino acid K392E, T411E, D399R and S400R mutations.

In one embodiment the heterodimerization approach described in WO2011/143545 is used alternatively. In one embodiment the amino acid modification according to WO2011/143545 is introduced in the CH3 domain of the heavy chain at a position selected from the group consisting of 368 and 409.

In one embodiment the heterodimerization approach described in WO2011/090762 which also uses the knob-into-hole technology described above is used alternatively. In one embodiment the CH3 domain of one heavy chain comprises an amino acid T366W mutation and the CH3 domain of the other heavy chain comprises an amino acid Y407A mutation. In one embodiment the CH3 domain of one heavy chain comprises an amino acid T366Y mutation and the CH3 domain of the other heavy chain of one heavy chain comprises an amino acid T366Y mutation and the CH3 domain of the other heavy chain comprises an amino acid T366Y mutation and the CH3 domain of the other heavy chain comprises an amino acid T366Y mutation.

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

In one embodiment the heterodimerization approach described in WO2009/089004 is used alternatively. In one embodiment the CH3 domain of one heavy chain comprises an amino acid substitution of K392 or N392 with a negatively-charged amino acid (in one embodiment glutamic acid (E) or aspartic acid (D); in a further embodiment a K392D or N392D mutation) and the CH3 domain of the other heavy chain comprises an amino acid substitution of D399, E356, D356, or E357 with a positively-charged amino acid (in one embodiment Lysine (K) or arginine (R), in a further embodiment a D399K, E356K, D356K or E357K substitution; and in an even further embodiment a D399K or E356K mutation). In a further embodiment the CH3 domain of the one heavy chain further comprises an amino acid substitution of K409 or R409 with a negatively-charged amino acid (in one embodiment glutamic acid (E) or aspartic acid (D); in a further embodiment a K409D or R409D mutation). In a further embodiment the CH3 domain of the one

15

10

5



heavy chain further or alternatively comprises an amino acid substitution of K439 and/or K370 with a negatively-charged amino acid (in one embodiment glutamic acid (E) or aspartic acid (D)).

In one embodiment the heterodimerization approach described in WO2007/147901 is used alternatively. In one embodiment the CH3 domain of one heavy chain comprises amino acid K253E, D282K and K322D mutations and the CH3 domain of the other heavy chain comprises amino acid D239K, E240K and K292D mutations.

In one embodiment the heterodimerization approach described in WO2007/110205 is used alternatively.

The terms "binding site" or "antigen-binding site" as used herein denotes the region(s) of an antibody molecule to which a ligand (e.g. the antigen or antigen fragment of it) actually binds and which is derived from an antibody. The antigen-binding site includes antibody heavy chain variable domains (VH) and/or an antibody light chain variable domain (VL), or pairs of VH/VL.

The antigen-binding sites that specifically bind to the desired antigen can be derived a) from known antibodies to the antigen or b) from new antibodies or antibody fragments obtained by de novo immunization methods using inter alia either the antigen protein or nucleic acid or fragments thereof, or by phage display.

An antigen-binding site of an antibody of the invention can contain six complementarity determining regions (CDRs) which contribute in varying degrees to the affinity of the binding site for antigen. There are three heavy chain variable domain CDRs (CDRH1, CDRH2 and CDRH3) and three light chain variable domain CDRs (CDRL1, CDRL2 and CDRL3). The extent of CDR and framework regions (FRs) is determined by comparison to a compiled database of amino acid sequences in which those regions have been defined according to variability among the sequences. Also included within the scope of the invention are functional antigen binding sites comprised of fewer CDRs (i.e., where binding specificity is determined by three, four or five CDRs). For example, less than a complete set of 6
CDRs may be sufficient for binding. In some cases, a VH or a VL domain will be sufficient.

5

10

Antibody specificity refers to selective recognition of the antibody for a particular epitope of an antigen. Natural antibodies, for example, are monospecific. The term "monospecific" antibody as used herein denotes an antibody that has one or more binding sites each of which bind to the same epitope of the same antigen.

5 Multispecific antibodies are e.g. bispecific, tri- or tetraspecific antibodies. Bispecific antibodies are antibodies which have two different antigen-binding specificities. Trispecific antibodies, accordingly, are antibodies which have three different antigen-binding specificities. Tetraspecific antibodies are antibodies which have four different antigen-binding specificities. In one preferred 10 embodiment of the invention the multispecific antibody is a bispecific antibody.

If an antibody has more than one specificity, the recognized epitopes may be associated with a single antigen or with more than one antigen.

The term "valent" as used within the current application denotes the presence of a specified number of binding sites in an antibody molecule. A natural antibody for example has two binding sites and is bivalent. As such, the term "trivalent" denotes the presence of three binding sites in an antibody molecule.

In one preferred embodiment of the invention the antibodies of the invention comprise immunoglobulin constant regions of one or more immunoglobulin classes. Immunoglobulin classes include IgG, IgM, IgA, IgD, and IgE isotypes and, in the case of IgG and IgA, their subtypes. In one preferred embodiment, an antibody of the invention has a constant domain structure of an IgG type antibody.

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

25 The term "chimeric antibody" refers to an antibody comprising a variable region, i.e., binding region, from one source or species and at least a portion of a constant region derived from a different source or species, usually prepared by recombinant DNA techniques. Chimeric antibodies comprising a murine variable region and a human constant region are preferred. Other preferred forms of "chimeric antibodies" encompassed by the present invention are those in which the constant region has been modified or changed from that of the original antibody to generate

15

the properties according to the invention, especially in regard to C1q binding and/or Fc receptor (FcR) binding. Such chimeric antibodies are also referred to as "class-switched antibodies". Chimeric antibodies are the product of expressed immunoglobulin genes comprising DNA segments encoding immunoglobulin variable regions and DNA segments encoding immunoglobulin constant regions. Methods for producing chimeric antibodies involve conventional recombinant DNA and gene transfection techniques are well known in the art. See, e.g., Morrison, S.L., et al., Proc. Natl. Acad. Sci. USA 81 (1984) 6851-6855; US 5,202,238 and US 5,204,244.

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

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germ line immunoglobulin sequences. Human antibodies are well-known in the state of the art (van Dijk, M.A., and van de Winkel, J.G., Curr. Opin. Chem. Biol. 5 (2001) 368-374). Human antibodies can also be produced in transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire or a selection of human antibodies in the absence of endogenous immunoglobulin production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (see, e.g., Jakobovits, A., et al., Proc. Natl. Acad. Sci. USA 90 (1993) 2551-2555; Jakobovits, A., et al., Nature 362 (1993) 255-258; Bruggemann, M., et al., Year Immunol. 7 (1993) 33-40). Human antibodies can also be produced in phage display libraries (Hoogenboom, H.R., and Winter, G., J. Mol. Biol. 227 (1992) 381-388; Marks, J.D., et al., J. Mol. Biol. 222 (1991) 581-597). The

5

25

techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole, et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); and Boerner, P., et al., J. Immunol. 147 (1991) 86-95). As already mentioned for chimeric and humanized antibodies according to the invention the term "human antibody" as used herein also comprises such antibodies which are modified in the constant region to generate the properties according to the invention, especially in regard to C1q binding and/or FcR binding, e.g. by "class switching" i.e. change or mutation of Fc parts (e.g. from IgG1 to IgG4 and/or IgG1/IgG4 mutation).

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

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

5

25

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

As used herein, the terms "binding", "that/which specifically binds", and "specifically binding" refer to the binding of the antibody to an epitope of the 15 antigen in an in vitro assay, preferably in an plasmon resonance assay (BIAcore®, GE-Healthcare Uppsala, Sweden) with purified wild-type antigen. The affinity of the binding is defined by the terms k_a (rate constant for the association of the antibody from the antibody/antigen complex), k_D (dissociation constant), and K_D (k_D/k_a) . In one embodiment "binding" or "that/which specifically binds to" means a binding affinity (K_D) of 10^{-8} mol/l or less, in one embodiment 10^{-8} M to 10^{-13} mol/l. 20 Thus, a multispecific antibody according to the invention specifically binds to each antigen for which it is specific with a binding affinity (K_D) of 10^{-8} mol/l or less, in one embodiment with a binding affinity (K_D) of 10^{-8} to 10^{-13} mol/l. In one embodiment the multispecific antibody specifically binds to its antigen with a 25 binding affinity (K_D) of 10⁻⁹ to 10⁻¹³ mol/l.

Binding of the antibody to the Fc γ RIII can be investigated by a BIAcore® assay (GE-Healthcare 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), k_D (dissociation constant), and K_D (k_D/ka).

30 The term "epitope" includes any polypeptide determinant capable of specific binding to an antibody. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific

10

5

25

three dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody.

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

In a further embodiment the multispecific antibody according to the invention is characterized in that said antibody is of human IgG1 subclass, or of human IgG1 subclass with the mutations L234A and L235A (numbering according to Kabat EU index).

10 In a further embodiment the multispecific antibody according to the invention is characterized in that said antibody is of human IgG2 subclass.

In a further embodiment the multispecific antibody according to the invention is characterized in that said antibody is of human IgG3 subclass.

In a further embodiment the multispecific antibody according to the invention is characterized in that said antibody is of human IgG4 subclass or, of human IgG4 subclass with the additional mutation S228P (numbering according to Kabat EU index).

In a further embodiment the multispecific antibody according to the invention is characterized in that it is of human IgG1 or human IgG4 subclass.

In a further embodiment the multispecific antibody according to the invention is characterized in being of human IgG1 subclass with the mutations L234A and L235A (numbering according to Kabat EU index).

In a further embodiment the multispecific antibody according to the invention is characterized in being of human IgG1 subclass with the mutations L234A, L235A and P329G (numbering according to Kabat EU index).

In a further embodiment the multispecific antibody according to the invention is characterized in being of human IgG4 subclass with the mutations S228P and L235E (numbering according to Kabat EU index).

- 31 -

In a further embodiment the multispecific antibody according to the invention is characterized in being of human IgG4 subclass with the mutations S228P, L235E and P329G (numbering according to Kabat EU index).

5

It has now been found that the multispecific antibodies according to the invention have improved characteristics, such as biological or pharmacological activity, pharmacokinetic properties or toxicity. They can be used e.g. for the treatment of diseases, such as cancer.

The term "constant region" as used within the current applications denotes the sum of the domains of an antibody other than the variable region. The constant region is 10 not involved directly in binding of an antigen, but exhibit various effector functions. Depending on the amino acid sequence of the constant region of their heavy chains, antibodies are divided in the classes: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses, such as IgG1, IgG2, IgG3, and IgG4, IgA1 and IgA2. The heavy chain constant regions that correspond 15 to the different classes of antibodies are called α , δ , ϵ , γ , and μ , respectively. The light chain constant regions (CL) which can be found in all five antibody classes are called κ (kappa) and λ (lambda). The "constant domains" as used herein are from human origin which is from a constant heavy chain region of a human antibody of the subclass IgG1, IgG2, IgG3, or IgG4 and/or a constant light chain kappa or lambda region. Such constant domains and regions are well known in the state of the art and e.g. described by Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991).

As used herein, the amino acid positions of all constant regions and domains of the 25 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 30 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, which is herein further clarified by referring to "numbering according to Kabat EU index" in this case).

While antibodies of the IgG4 subclass show reduced Fc receptor (FcγRIIIa) binding, antibodies of other IgG subclasses show strong binding. However Pro238, Asp265, Asp270, Asn297 (loss of Fc carbohydrate), Pro329, Leu234, Leu235, Gly236, Gly237, Ile253, Ser254, Lys288, Thr307, Gln311, Asn434, and His435 (numberings according to Kabat EU index) are residues which, if altered, provide also reduced Fc receptor binding (Shields, R.L., et al., J. Biol. Chem. 276 (2001) 6591-6604; Lund, J., et al., FASEB J. 9 (1995) 115-119; Morgan, A., et al., Immunology 86 (1995) 319-324; EP 0 307 434).

In one embodiment an antibody according to the invention has a reduced FcR binding compared to an IgG1 antibody. Thus, the parent antibody is in regard to FcR binding of IgG4 subclass or of IgG1 or IgG2 subclass with a mutation in S228, L234, L235 and/or D265, and/ or contains the PVA236 mutation (numberings according to Kabat EU index). In one embodiment the mutations in the parent antibody are S228P, L234A, L235A, L235E and/or PVA236 (numberings according to Kabat EU index). In another embodiment the mutations in the parent antibody are in IgG4 S228P and in IgG1 L234A and L235A (numberings according to Kabat EU index).

20 The constant region of an antibody is directly involved in ADCC (antibodycytotoxicity) and CDC dependent cell-mediated (complement-dependent cytotoxicity). Complement activation (CDC) is initiated by binding of complement factor C1q to the constant region of most IgG antibody subclasses. Binding of C1q to an antibody is caused by defined protein-protein interactions at the so called 25 binding site. Such constant region binding sites are known in the state of the art and described e.g. by Lukas, T.J., et al., J. Immunol. 127 (1981) 2555-2560; Bunkhouse, R. and Cobra, J.J., Mol. Immunol. 16 (1979) 907-917; Burton, D.R., et al., Nature 288 (1980) 338-344; Thomason, J.E., et al., Mol. Immunol. 37 (2000) 995-1004; Idiocies, E.E., et al., J. Immunol. 164 (2000) 4178-4184; Hearer, M., et 30 al., J. Virol. 75 (2001) 12161-12168; Morgan, A., et al., Immunology 86 (1995) 319-324; and EP 0 307 434. Such constant region binding sites are, e.g., characterized by the amino acids L234, L235, D270, N297, E318, K320, K322, P331, and P329 (numbering according to Kabat EU index).

5

10

WO 2015/150447

The term "antibody-dependent cellular cytotoxicity (ADCC)" refers to lysis of human target cells by an antibody according to the invention in the presence of effector cells. ADCC is measured preferably by the treatment of a preparation of antigen expressing cells with an antibody according to the invention in the presence of effector cells such as freshly isolated PBMC or purified effector cells from buffy coats, like monocytes or natural killer (NK) cells or a permanently growing NK cell line.

The term "complement-dependent cytotoxicity (CDC)" denotes a process initiated by binding of complement factor C1q to the Fc part of most IgG antibody subclasses. Binding of C1q to an antibody is caused by defined protein-protein interactions at the so called binding site. Such Fc part binding sites are known in the state of the art (see above). Such Fc part binding sites are, e.g., characterized by the amino acids L234, L235, D270, N297, E318, K320, K322, P331, and P329 (numbering according to Kabat EU index). Antibodies of subclass IgG1, IgG2, and IgG3 usually show complement activation including C1q and C3 binding, whereas IgG4 does not activate the complement system and does not bind C1q and/or C3.

Cell-mediated effector functions of monoclonal antibodies can be enhanced by engineering their oligosaccharide component as described in Umana, P., et al., Nature Biotechnol. 17 (1999) 176-180, and US 6,602,684. IgG1 type antibodies, the most commonly used therapeutic antibodies, are glycoproteins that have a 20 conserved N-linked glycosylation site at Asn297 in each CH2 domain. The two complex biantennary oligosaccharides attached to Asn297 are buried between the CH2 domains, forming extensive contacts with the polypeptide backbone, and their presence is essential for the antibody to mediate effector functions such as antibody 25 dependent cellular cytotoxicity (ADCC) (Lifely, M., R., et al., Glycobiology 5 (1995) 813-822; Jefferis, R., et al., Immunol. Rev. 163 (1998) 59-76; Wright, A., and Morrison, S.L., Trends Biotechnol. 15 (1997) 26-32). Umana, P., et al., Nature Biotechnol. 17 (1999) 176-180 and WO 99/54342 showed that overexpression in Chinese hamster ovary (CHO) cells of $\beta(1,4)$ -N-acetylglucosaminyltransferase III 30 ("GnTIII"), a glycosyltransferase catalyzing the formation of bisected oligosaccharides, significantly increases the in vitro ADCC activity of antibodies. Alterations in the composition of the Asn297 carbohydrate or its elimination affect also binding to FcyR and C1q (Umana, P., et al., Nature Biotechnol. 17 (1999) 176-180; Davies, J., et al., Biotechnol. Bioeng. 74 (2001) 288-294; Mimura, Y., et al., J.

10

5

Biol. Chem. 276 (2001) 45539-45547; Radaev, S., et al., J. Biol. Chem. 276 (2001) 16478-16483; Shields, R.L., et al., J. Biol. Chem. 276 (2001) 6591-6604; Shields, R.L., et al., J. Biol. Chem. 277 (2002) 26733-26740; Simmons, L.C., et al., J. Immunol. Methods 263 (2002) 133-147).

5 Methods to enhance cell-mediated effector functions of monoclonal antibodies are WO 2005/018572, WO 2006/116260, WO 2006/114700, reported e.g. in WO 1997/028267. WO 2004/065540. WO 2005/011735, WO 2005/027966, US 2006/0134709. US 2005/0054048, US 2005/0152894. WO 2003/035835. WO 2000/061739.

10 In one preferred embodiment of the invention, the multispecific antibody is glycosylated (if it comprises an Fc part of IgG1, IgG2, IgG3 or IgG4 subclass, preferably of IgG1 or IgG3 subclass) with a sugar chain at Asn297 whereby the amount of fucose within said sugar chain is 65% or lower (numbering according to Kabat EU index). In another embodiment is the amount of fucose within said sugar 15 chain is between 5% and 65%, preferably between 20% and 40%. "Asn297" according to the invention means amino acid asparagine located at about position 297 in the Fc region. Based on minor sequence variations of antibodies, Asn297 can also be located some amino acids (usually not more than +3 amino acids) upstream or downstream of position 297, i.e. between position 294 and 300. In one 20 embodiment the glycosylated antibody according to the invention the IgG subclass is of human IgG1 subclass, of human IgG1 subclass with the mutations L234A and L235A or of IgG3 subclass. In a further embodiment the amount of Nglycolylneuraminic acid (NGNA) is 1% or less and/or the amount of N-terminal alpha-1,3-galactose is 1% or less within said sugar chain. The sugar chain 25 preferably exhibits the characteristics of N-linked glycans attached to Asn297 of an antibody recombinantly expressed in a CHO cell.

The term "the sugar chains show characteristics of N-linked glycans attached to Asn297 of an antibody recombinantly expressed in a CHO cell" denotes that the sugar chain at Asn297 of the parent antibody according to the invention has the same structure and sugar residue sequence except for the fucose residue as those of the same antibody expressed in unmodified CHO cells, e.g. as those reported in WO 2006/103100.

The term "NGNA" as used within this application denotes the sugar residue N-glycolylneuraminic acid.

Glycosylation of human IgG1 or IgG3 occurs at Asn297 as core fucosylated biantennary complex oligosaccharide glycosylation terminated with up to two Gal 5 residues. Human constant heavy chain regions of the IgG1 or IgG3 subclass are reported in detail by Kabat, E., A., et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991), and by Brüggemann, M., et al., J. Exp. Med. 166 (1987) 1351-1361; Love, T., W., et al., Methods Enzymol. 178 (1989) 515-527. These structures are 10 designated as G0, G1 (α -1,6- or α -1,3-), or G2 glycan residues, depending from the amount of terminal Gal residues (Raju, T., S., Bioprocess Int. 1 (2003) 44-53). CHO type glycosylation of antibody Fc parts is e.g. described by Routier, F., H., Glycoconjugate J. 14 (1997) 201-207. Antibodies which are recombinantly expressed in non-glycomodified CHO host cells usually are fucosylated at Asn297 15 in an amount of at least 85%. The modified oligosaccharides of the antibody may be hybrid or complex. Preferably the bisected, reduced/not-fucosylated oligosaccharides are hybrid. In another embodiment, the bisected, reduced/notfucosylated oligosaccharides are complex.

According to the invention "amount of fucose" means the amount of said sugar
 within the sugar chain at Asn297, related to the sum of all glycostructures attached to Asn297 (e.g. complex, hybrid and high mannose structures) measured by MALDI-TOF mass spectrometry and calculated as average value. The relative amount of fucose is the percentage of fucose-containing structures related to all glycostructures identified in an N-Glycosidase F treated sample (e.g. complex, hybrid and oligo- and high-mannose structures, resp.) by MALDI-TOF.

Antibodies according to the invention may bind to a variety of antigens. In one embodiment of the invention, neither the first antigen nor the second antigen is an activating T cell antigen. In one embodiment of the invention, neither the first antigen nor the second antigen is CD3. In one embodiment, the antibody does not specifically bind to an activating T cell antigen. In one embodiment, the antibody does not specifically bind to CD3.

30

In one embodiment of the invention the first or the second antigen is human TWEAK. In one embodiment of the invention the first or the second antigen is

human IL17. In one embodiment of the invention the first antigen is human TWEAK and the second antigen is human IL17. In one embodiment of the invention the first antigen is human IL17 and the second antigen is human TWEAK.

5 Human TWEAK (UniProtKB O43508, TNF-related weak inducer of apoptosis) is a cell surface associated type II transmembrane protein. TWEAK is described in Chicheportiche, Y., et al., J. Biol. Chem. 272 (1997) 32401-32410; Marsters, S.A., et al., Curr. Biol. 8 (1998) 525-528; Lvnch, C.N., et al., J. Biol. Chem. 274 (1999) 8455-8459. The active form of TWEAK is a soluble homotrimer. Human and 10 murine TWEAK show 93 % sequence identity in receptor binding domain. The TWEAK receptor Fn14 (fibroblast growth factor inducible 14 kDa protein) is a 129 aa type I transmembane protein consisting of one single cystein rich domain in ligand binding domain. Signaling of TWEAK occurs via NF-KB pathway activation. TWEAK mRNA is expressed in a variety of tissues and found in most 15 major organs like heart, brain, skeletal muscle, and pancreas, tissues related to the immune system like spleen, lymph nodes, and thymus. Fn14 mRNA has been detected in heart, brain, lung, placenta, vascular EC and smooth muscle cells. TWEAK-null and Fnl4-null knockout mice are viable, healthy and fertile and have more natural killer cells and display an enhanced innate inflammatory response. 20 TWEAK is involved in apoptosis, proliferation, angiogenesis, ischemic penumbra, cerebral edema, multiple sclerosis.

Human IL-17 (also named IL17-A; CTLA-8, Swiss Prot Q16552, IL17) is a proinflammatory cytokine produced by a subset of helper T cells (called Th17) that has been implicated in the pathogenesis of MS. IL-17A plays a role in the induction of other inflammatory cytokines, chemokines and adhesion molecules. Treatment of animals with IL-17A neutralizing antibodies decreases disease incidence and severity in autoimmune encephalomyelitis (Komiyama, Y. et al., J. Immunol. 177 (2006) 566-573). IL-17A is over-expressed in the cerebrospinal fluid of MS patients (Hellings, P.W. et al., Am. J. Resp. Cell Mol. Biol. 28 (2003) 42-50; 30 Matusevicius, D. et al., Multiple Sclerosis 5 (1999) 101-104; WO 2005/051422). In addition, IL-17A neutralizing antibodies reduce severity and incidence of mouse RA model of collagen induced arthritis, and high levels of IL-17A can be detected in the synovial fluid of inflamed joints from RA patients (Ziolkowska, M. et al., J. Immunol. 164 (2000) 2832-2838; Kotake, S., et al., J. Clin. Invest. 103 (1999) 1345-1352; Hellings, P.W. et al., Am. J. Resp. Cell Mol. Biol. 28 (2003) 42-50).

The antibody according to the invention is produced by recombinant means. Thus, one aspect of the current invention is a nucleic acid encoding the antibody 5 according to the invention and a further aspect is a cell comprising said nucleic acid encoding an antibody according to the invention. Methods for recombinant production are widely known in the state of the art and comprise protein expression in prokaryotic and eukaryotic cells with subsequent isolation of the antibody and usually purification to a pharmaceutically acceptable purity. For the expression of 10 the antibodies as aforementioned in a host cell, nucleic acids encoding the respective modified light and heavy chains are inserted into expression vectors by standard methods. Expression is performed in appropriate prokaryotic or eukaryotic host cells like CHO cells, NS0 cells, SP2/0 cells, HEK293 cells, COS cells, PER.C6 cells, yeast, or E.coli cells, and the antibody is recovered from the cells 15 (supernatant or cells after lysis). General methods for recombinant production of antibodies are well-known in the state of the art and described, for example, in the review articles of Makrides, S.C., Protein Expr. Purif. 17 (1999) 183-202; Geisse, S., et al., Protein Expr. Purif. 8 (1996) 271-282; Kaufman, R.J., Mol. Biotechnol. 16 (2000) 151-161; Werner, R.G., Drug Res. 48 (1998) 870-880.

20 Antibodies produced by host cells may undergo post-translational cleavage of one or more, particularly one or two, amino acids from the C-terminus of the heavy chain. Therefore an antibody produced by a host cell by expression of a specific nucleic acid molecule encoding a full-length heavy chain may include the fulllength heavy chain, or it may include a cleaved variant of the full-length heavy 25 chain (also referred to herein as a cleaved variant heavy chain). This may be the case where the final two C-terminal amino acids of the heavy chain are glycine (G446) and lysine (K447, numbering according to Kabat EU index).

Therefore, amino acid sequences of heavy chains including CH3 domains are denoted herein without C-terminal glycine-lysine dipeptide if not indicated otherwise.

In one embodiment, an antibody comprising a heavy chain including a CH3 domain, as specified herein, comprises an additional C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to EU index of Kabat). In one

embodiment, an antibody comprising a heavy chain including a CH3 domain, as specified herein, comprises an additional C-terminal glycine residue (G446, numbering according to EU index of Kabat).

Compositions of the invention, such as the pharmaceutical compositions described 5 herein, comprise a population of antibodies of the invention. The population of antibodies may comprise antibodies having a full-length heavy chain and antibodies having a cleaved variant heavy chain. The population of antibodies may consist of a mixture of antibodies having a full-length heavy chain and antibodies having a cleaved variant heavy chain, wherein at least 50%, at least 60%, at least 10 70%, at least 80% or at least 90% of the antibodies have a cleaved variant heavy chain.

In one embodiment, a composition comprising a population of antibodies of the invention comprises an antibody comprising a heavy chain including a CH3 domain, as specified herein, with an additional C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to EU index of Kabat). In one embodiment, a composition comprising a population of antibodies of the invention comprises an antibody comprising a heavy chain including a CH3 domain, as specified herein, with an additional C-terminal glycine residue (G446, numbering according to EU index of Kabat).

20 In one embodiment, such a composition comprises a population of antibodies comprised of antibodies comprising a heavy chain including a CH3 domain, as specified herein; antibodies comprising a heavy chain including a CH3 domain, as specified herein, with an additional C-terminal glycine residue (G446, numbering according to EU index of Kabat); and antibodies comprising a heavy chain 25 including a CH3 domain, as specified herein, with an additional C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to EU index of Kabat).

The multispecific antibodies according to the invention are suitably separated from the culture medium by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography. DNA and RNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures. The hybridoma cells can serve as a source of such DNA and RNA.

15

Once isolated, the DNA may be inserted into expression vectors, which are then transfected into host cells such as HEK 293 cells. CHO cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of recombinant monoclonal antibodies in the host cells.

5 Amino acid sequence variants (or mutants) of the multispecific antibody are prepared by introducing appropriate nucleotide changes into the antibody DNA, or by nucleotide synthesis. Such modifications can be performed, however, only in a very limited range, e.g. as described above. For example, the modifications do not alter the above mentioned antibody characteristics such as the IgG isotype and antigen binding, but may further improve the yield of the recombinant production, protein stability or facilitate the purification. In certain embodiments, antibody variants having one or more conservative amino acid substitutions are provided.

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

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

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

- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

20 Table 1 – Amino acids with specific properties

Amino Acid	3-Letter	1-	Side-chain	Side-chain
		Letter	polarity	charge (pH 7.4)
Alanine	Ala	А	nonpolar	neutral
Arginine	Arg	R	basic polar	positive
Asparagine	Asn	N	polar	neutral
Aspartic acid	Asp	D	acidic polar	negative
Cysteine	Cys	С	nonpolar	neutral
Glutamic acid	Glu	Е	acidic polar	negative

10

Amino Acid	3-Letter	1-	Side-chain	Side-chain
		Letter	polarity	charge (pH 7.4)
Glutamine	Gln	Q	polar	neutral
Glycine	Gly	G	nonpolar	neutral
Histidine	His	Н	basic polar	positive (10%)
				neutral (90%)
Isoleucine	Ile	Ι	nonpolar	neutral
Leucine	Leu	L	nonpolar	neutral
Lysine	Lys	К	basic polar	positive
Methionine	Met	Μ	nonpolar	neutral
Phenylalanine	Phe	F	nonpolar	neutral
Proline	Pro	Р	nonpolar	neutral
Serine	Ser	S	polar	neutral
Threonine	Thr	Т	polar	neutral
Tryptophan	Trp	W	nonpolar	neutral
Tyrosine	Tyr	Y	polar	neutral
Valine	Val	V	nonpolar	neutral

- 40 -

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

Expression in NS0 cells is described by, e.g., Barnes, L.M., et al., Cytotechnology 32 (2000) 109-123; Barnes, L.M., et al., Biotech. Bioeng. 73 (2001) 261-270.
Transient expression is described by, e.g., Durocher, Y., et al., Nucl. Acids. Res. 30 (2002) E9. Cloning of variable domains is described by Orlandi, R., et al., Proc. Natl. Acad. Sci. USA 86 (1989) 3833-3837; Carter, P., et al., Proc. Natl. Acad. Sci.

5

10

- 41 -

USA 89 (1992) 4285-4289; and Norderhaug, L., et al., J. Immunol. Methods 204 (1997) 77-87. A preferred transient expression system (HEK 293) is described by Schlaeger, E.-J., and Christensen, K., in Cytotechnology 30 (1999) 71-83 and by Schlaeger, E.-J., J. Immunol. Methods 194 (1996) 191-199.

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

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

20 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 25 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 30 (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).

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

One embodiment of the invention is the multispecific antibody according to the invention for use in the treatment of cancer.

Another aspect of the invention is said pharmaceutical composition for use in the treatment of cancer.

15 Another aspect of the invention is the use of an antibody according to the invention for the manufacture of a medicament for the treatment of cancer.

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

20 One embodiment of the invention is the multispecific antibody according to the invention for use in the treatment of inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular 25 injury.

Another aspect of the invention is said pharmaceutical composition for use in the treatment of inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

10

30

WO 2015/150447

5

10

Another aspect of the invention is the use of an antibody according to the invention for the manufacture of a medicament for the treatment of inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

Another aspect of the invention is method of treatment of a patient suffering from inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury, by administering an antibody according to the invention to a patient in the need of such treatment.

- As used herein, "pharmaceutical carrier" includes any and all solvents, dispersion 15 media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g. by injection or infusion).
- A composition of the present invention can be administered by a variety of 20 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.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intra-articular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

- 5 The term cancer as used herein refers to proliferative diseases, such as lymphomas, lymphocytic leukemias, lung cancer, non small cell lung (NSCL) cancer, bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric 10 cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, 15 cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme. astrocytomas, schwanomas, ependymonas, medulloblastomas. 20 meningiomas, squamous cell carcinomas, pituitary adenoma and Ewings sarcoma, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers.
- These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the

pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

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

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

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

The term "transformation" as used herein refers to process of transfer of a vectors/nucleic acid into a host cell. If cells without formidable cell wall barriers are used as host cells, transfection is carried out e.g. by the calcium phosphate precipitation method as described by Graham and Van der Eh, Virology 52 (1978) 546ff. However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used. If prokaryotic cells or cells which contain substantial cell wall constructions are used, e.g. one method of transfection is calcium treatment using calcium chloride as described by Cohen, F.N, et al., PNAS 69 (1972) 7110 et seq. As used herein, "expression" refers to the process by which a nucleic acid is transcribed into mRNA and/or to the process by which the transcribed mRNA (also referred to as transcript) is subsequently being translated into peptides, polypeptides, or proteins. The transcripts and the encoded polypeptides are collectively referred to as gene product. If the polynucleotide is derived from genomic DNA, expression in a eukaryotic cell may include splicing of the mRNA.

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

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

In the following specific embodiments of the invention are listed:

- 1. A multispecific antibody, comprising:
 - a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and
 - b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

wherein

i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein

10

5

15

20

30

WO 2015/150447

- 47 -

in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index); or

- ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at positions 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).
 - 2. A multispecific antibody, comprising:
 - a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and
 - b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and
- 20 wherein

15

- i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).
- 3. The multispecific antibody according to embodiment 1 or 2,

20

25

30

wherein in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

4. The multispecific antibody according to embodiment 1 or 2,

- 10 wherein in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 is substituted independently by glutamic acid (E) or aspartic
 15 acid (D) (numbering according to Kabat EU index).
 - 5. The multispecific antibody according to embodiment 4,
 - wherein in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat); and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).
 - 6. The multispecific antibody according to embodiment 1 or 2,
 - wherein in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K) or arginine (R) (numbering according to Kabat), and wherein in the constant

30

domain CH1 of the first heavy chain under a) the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

- 7. The multispecific antibody according to embodiment 1 or 2,
- 5 wherein in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)); and wherein in the constant domain CH1 of the second heavy chain under b) the 10 amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).
 - 8. The multispecific antibody according to embodiment 1 or 2,
- wherein in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (in one preferred embodiment independently by lysine (K) or arginine (R)) (numbering according to Kabat), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at position 147 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

9. The multispecific antibody according to embodiment 8,

wherein in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of second the heavy chain under b) the amino acid at position 147 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

- 10. The multispecific antibody according to embodiment 1 or 2,
- wherein in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K). arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).
- 10 The multispecific antibody according to embodiment 5, 11.

wherein in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) (numbering according to Kabat),

- 15 and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).
- 20 12. The multispecific antibody according to embodiment 9,

wherein in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) (numbering according to Kabat),

25 and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

- 13. The multispecific antibody according to any one of the preceding embodiments, wherein the constant domains CL of the first light chain under a) and the second light chain under b) are of kappa isotype.
- 14. The multispecific antibody according to any one of embodiments 1 to 12, wherein the constant domain CL of the first light chain under a) is of lambda isotype and the constant domain CL of the second light chain under b) is of kappa isotype.
 - 15. The multispecific antibody according to any one of embodiments 1 to 12, wherein the constant domains CL of the first light chain under a) and the second light chain under b) are of lambda isotype.
 - 16. The multispecific antibody according to any one of the preceding embodiments wherein in the constant domain CL of either the first light chain under a) or the second light chain under b), in which the amino acid at position 124 is <u>not</u> substituted independently by lysine (K), arginine (R) or histidine (H) and which is of kappa isotype, the amino acid at position 124 is substituted independently by glutamic acid (E) or aspartic acid (D) (in one preferred embodiment by glutamic acid (E)) (numbering according to Kabat).
 - 17. The antibody according to any one of the preceding embodiments, characterized in that

a first CH3 domain of the first heavy chain of the antibody under a) and a 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,

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

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

so that within the original interface of the CH3 domain of the one30heavy chain that meets the original interface of the CH3 domain
of the other heavy chain within the multispecific antibody,

an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a

5

15

20

25

10

protuberance within the interface of the CH3 domain of the one heavy chain which is positionable in a cavity within the interface of the CH3 domain of the other heavy chain and ii) the CH3 domain of the other heavy chain is altered,

so that within the original interface of the CH3 domain of the other heavy chain that meets the original interface of the CH3 domain of the one heavy chain within the multispecific antibody an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the interface of the CH3 domain of the other heavy chain within which a protuberance within the interface of the CH3 domain of the one heavy chain is positionable.

15 18. The antibody according to embodiment 17, characterized in that the said amino acid residue having a larger side chain volume is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W), and said amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine
20 (S), threonine (T) and valine (V).

19. The antibody according to embodiments 17 or 18, characterized in that 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.

- 20. A multispecific antibody according to any one of the preceding embodiments wherein the antibody is bispecific.
- 21. A multispecific antibody according to any one of the preceding embodiments that specifically binds to human TWEAK and that specifically binds to human IL17, wherein

A) the multispecific antibody comprises a variable heavy chain domain (VH) of SEQ ID NO:24, and a variable light chain domain (VL) of SEQ ID NO:25; and

30

B) the multispecific antibody comprises a variable heavy chain domain (VH) of SEQ ID NO:26, and a variable light chain domain (VL) of SEQ ID NO:27.

- 22. A bispecific antibody that comprises
- a) the first light chain and the first heavy chain of a first antibody which specifically binds to a human TWEAK, which comprises a variable heavy chain domain (VH) of SEQ ID NO:24, and a variable light chain domain (VL) of SEQ ID NO:25; and
- b) the second light chain and the second heavy chain of a second antibody which specifically binds to a human IL-17, which comprises a variable heavy chain domain (VH) of SEQ ID NO:26, and a variable light chain domain (VL) of SEQ ID NO:27; wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other, and
- 15 wherein
 - i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K) or arginine (R), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

23. The bispecific antibody according to embodiment 21, wherein

in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K) or arginine (R) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or Histidine (H) (numbering according to Kabat); and wherein in the constant domain CH1 of the first heavy chain under a)
the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the

5

10

- 54 -

amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

An antibody according to any one of embodiments 21 to 23 for use in the 24. treatment of cancer, or inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

- Use of an antibody according to any one of embodiments 21 to 23 for 25. manufacture of a medicament for the treatment of cancer, or inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.
- 15 The multispecific antibody according to any one of embodiments 1 to 23, 26. characterized in that it is of human IgG1 or human IgG4 subclass.
 - The multispecific antibody according to any one of embodiments 1 to 23 and 27. 26, characterized in being of human IgG1 subclass with the mutations L234A and L235A (numbering according to Kabat EU index).
- 20 28. The multispecific antibody according to any one of embodiments 1 to 23 and 26 to 27, characterized in being of human IgG1 subclass with the mutations L234A, L235A and P329G (numbering according to Kabat EU index).
 - 29. The multispecific antibody according to any one of preceding embodiments 1 to 23 and 26, characterized in being of human IgG4 subclass with the mutations S228P and L235E (numbering according to Kabat EU index).
 - The multispecific antibody according to any one of embodiments 1 to 23, and 30. 26 to 29, characterized in being of human IgG4 subclass with the mutations S228P, L235E and P329G (numbering according to Kabat EU index).
 - 31. A method for the preparation of a multispecific antibody according to any one of embodiments 1 to 23 and 26 to 30,

10

25

30

comprising the steps of

A) transforming a host cell with vectors comprising nucleic acid molecules encoding

- a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and
- b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

10 wherein

5

15

20

- i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index); or
- ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at positions 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index);
 - B) culturing the host cell under conditions that allow synthesis of said antibody molecule; and
 - C) recovering said antibody molecule from said culture.

- Nucleic acid encoding the amino acid sequences of a multispecific antibody 32. according to any one of embodiments 1 to 23 and 26 to 30.
- Expression vector containing the nucleic acid according to embodiment 32 33. capable of expressing said nucleic acid in a host cell.
- 5 34. A host cell comprising a vector according to embodiment 33.
 - 35. A composition comprising the antibody according to any one of embodiments 1 to 23 and 26 to 30.
 - A pharmaceutical composition comprising an antibody according to any one 36. of embodiments 1 to 23 and 26 to 30 and at least one pharmaceutically acceptable excipient.
 - 37. A method for the treatment of a patient in need of therapy, characterized by administering to the patient a therapeutically effective amount of an antibody according to any one of embodiments 1 to 23 and 26 to 30.
 - 38. A method for the reduction of side products of multispecific antibodies,

15 comprising the steps of

A) transforming a host cell with vectors comprising nucleic acid molecules encoding

- a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and
- 20 b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and
 - wherein the following substitions are included for reducing the side products of the multispecific antibody:
 - i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred

10

10

15

20

25

embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index); or

- ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at positions 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index);
- B) culturing the host cell under conditions that allow synthesis of said antibody molecule; and

C) recovering said antibody molecule from said culture.

39. A method for the reduction of side products of multispecific antibodies

comprising the steps of

A) transforming a host cell with vectors comprising nucleic acid molecules encoding

- a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and
- b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

wherein the following substitions are included,

i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or WO 2015/150447

5

10

- 58 -

Histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K), arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index); or

- ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or Histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K), arginine (R)), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at positions 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index),
- 15 B) culturing the host cell under conditions that allow synthesis of said antibody molecule; and

C) recovering said antibody molecule with a reduced side product profile from said culture.

- 40. A method for the reduction of side products of multispecific antibodies
- 20 comprising the steps of

A) transforming a host cell with vectors comprising nucleic acid molecules encoding

- a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and
- b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

wherein the following substitions are included,

i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or Histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K), arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index),

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

C) recovering said antibody molecule with a reduced side product profile from said culture.

41. Use of the following substitions for reducing the formation of side products (or for reducing the side product profile) of a multispecific antibody:

i) in the constant domain CL of a first light chain under a) substituting the amino acid at position 124 independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of a first heavy chain under a) substituting the amino acid at position 147 or the amino acid at position 213 independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index); or

ii) in the constant domain CL of a second light chain under
b) substituting the amino acid at position 124 independently
by lysine (K), arginine (R) or histidine (H) (numbering
according to Kabat) (in one preferred embodiment
independently by lysine (K)or arginine (R)), and in the
constant domain CH1 of a second heavy chain under b)
substituting the amino acid at positions 147 or the amino
acid at position 213 independently by glutamic acid (E) or

5

10

15

aspartic acid (D) (numbering according to Kabat EU index):

wherein the multispecific antibody comprises

- a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and
 - b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other.
- 42. Use of the following substitions for reducing the formation of side products (or for reducing the side product profile) of a multispecific antibody:

i) in the constant domain CL of a first light chain under a) substituting the amino acid at position 124 independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of a first heavy chain under a) substituting the amino acid at position 147 or the amino acid at position 213 independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index);

wherein the multispecific antibody comprises

- a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and
- 25 b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other.

10

15

20

- The multispecific antibody according to any one of embodiments 1 to 16, 43. and 20 to 23, wherein the antibody comprises at least two Fab fragments, wherein the first Fab fragment comprises at least one antigen binding site specific for a first antigen; and the second Fab fragment comprises at least one antigen binding site specific for a second antigen, wherein in the second Fab fragment the variable domains VL and VH in the second light chain and second heavy chain are replaced by each other; and wherein the multispecific antibody is devoid of an Fc domain.
- 44. The multispecific antibody according to embodiment 43, wherein the antibody comprises two to four Fab fragments.
- The multispecific antibody according to embodiment 43 or 44, wherein the 45. antibody specifically binds to human Ang-2 and VEGF.
- 46. A method of producing an antibody comprising culturing the host cell of embodiment 34 so that the antibody is produced.
- 47. The method of embodiment 46, further comprising recovering the antibody from the host cell.

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

Description of the Amino acid Sequences

	SEQ ID NO:1	light chain (LC) <ang-2> wild type (wt)</ang-2>
	SEQ ID NO:2	heavy chain (HC) <ang-2> wild type (wt)</ang-2>
	SEQ ID NO:3	heavy chain (HC) <vegf> with VH-VL exchange wild type (wt)</vegf>
25	SEQ ID NO:4	light chain (LC) <vegf> with VH-VL exchange wild type (wt)</vegf>
	SEQ ID NO:5	light chain (LC) <ang-2> with Q124K substitution</ang-2>
	SEQ ID NO:6	heavy chain (HC) < Ang-2> with K147E substitution
	SEQ ID NO:7	heavy chain (HC) < Ang-2> with K213E substitution
	SEQ ID NO:8	light chain (LC) <ang-2> with E123K substitution</ang-2>
30	SEQ ID NO:9	light chain (LC) <ang-2> with Q124K substitution and E123K</ang-2>
		substitution

10

5

15

	SEQ ID NO:10	heavy chain (HC) <ang-2> with K147E substitution and K213E substitution</ang-2>
	SEQ ID NO:11	light chain (LC) <ang-2> with Q124R substitution and E123K substitution</ang-2>
5	SEQ ID NO:12	light chain (LC) <vegf> with Q124E substitution</vegf>
	SEQ ID NO:13	light chain (LC) <ang-2> with E124K substitution and E123K substitution</ang-2>
	SEQ ID NO:14	heavy chain (HC) <ang-2> with K147E substitution and K213D substitution</ang-2>
10	SEQ ID NO:15	light chain (LC) <il-17> wild type (wt)</il-17>
	SEQ ID NO:16	heavy chain (HC) <il-17> wild type (wt)</il-17>
	SEQ ID NO:17	heavy chain (HC) <tweak> with VH-VL exchange wild type (wt)</tweak>
15	SEQ ID NO:18	light chain (LC) <tweak> with VH-VL exchange wild type (wt)</tweak>
	SEQ ID NO:19	light chain (LC) <il-17> with Q124K substitution and E123R substitution</il-17>
	SEQ ID NO:20	heavy chain (HC) <il-17> with K147E substitution and K213E substitution</il-17>
20	SEQ ID NO:21	light chain (LC) <tweak> with Q124E substitution</tweak>
	SEQ ID NO:22	heavy chain (HC) <il-17> with K147E substitution and K213D substitution</il-17>
	SEQ ID NO:23	light chain (LC) <il-17> with Q124K substitution and E123K substitution</il-17>
25	SEQ ID NO:24	variable heavy chain domain VH <tweak> 305-HC4</tweak>
	SEQ ID NO:25	variable light chain domain VL <tweak>305-LC2</tweak>
	SEQ ID NO:26	variable heavy chain domain VH <il-17> HC136</il-17>
	SEQ ID NO:27	variable light chain domain VL <il-17> LC136</il-17>
	SEQ ID NO: 28	heavy chain (HC) <tweak> with VH-VL exchange wild type</tweak>
30		(wt) (comprising terminal GK dipeptide)
	SEQ ID NO: 29	heavy chain (HC) <il-17> with K147E substitution and K213E</il-17>
		substitution (comprising terminal GK dipeptide)
	SEQ ID NO: 30	heavy chain (HC) <il-17> with K147E substitution and K213D</il-17>
		substitution (comprising terminal GK dipeptide)

WO 2015/150447

- SEQ ID NO: 31 heavy chain (HC) <Ang-2> wild type (wt) (comprising terminal GK dipeptide)
 - SEQ ID NO: 32 heavy chain (HC) <VEGF> with VH-VL exchange wild type (wt) (comprising terminal GK dipeptide)
- 5 SEQ ID NO: 33 heavy chain (HC) <Ang-2> with K147E substitution (comprising terminal GK dipeptide)
 - SEQ ID NO: 34 heavy chain (HC) <Ang-2> with K213E substitution (comprising terminal GK dipeptide)
 - **SEQ ID NO: 35** heavy chain (HC) <Ang-2> with K147E substitution and K213E substitution (comprising terminal GK dipeptide)
 - **SEQ ID NO: 36** heavy chain (HC) <Ang-2> with K147E substitution and K213D substitution (comprising terminal GK dipeptide)
 - SEQ ID NO: 37 heavy chain (HC) <IL-17> wild type (wt) (comprising terminal GK dipeptide)
- 15 SEQ ID NO: 38 Fab₂-CrossFab heavy chain (HC) including two heavy chains (HC) <Ang-2> wild type (wt) coupled to one heavy chain (HC) <VEGF> with VH-VL exchange wild type (wt) via glycineserine-linkers
 - SEQ ID NO: 39 Fab₂-CrossFab heavy chain (HC) including two heavy chains (HC) <Ang-2> with K147E and K213E substitutions coupled to one heavy chain (HC) <VEGF> with VH-VL exchange wild type (wt) via glycine-serine-linkers
 - SEQ ID NO: 40 CrossFab-Fab heavy chain (HC) including one heavy chain (HC) <VEGF> with VH-VL exchange wild type (wt) coupled to one heavy chain (HC) <Ang-2> with K147E and K213E substitutions via glycine-serine-linkers
 - SEQ ID NO: 41 CrossFab-Fab heavy chain (HC) including one heavy chain (HC) <VEGF> with VH-VL exchange wild type (wt) coupled to one heavy chain (HC) <Ang-2> with K147E and K213E substitutions via glycine-serine-linkers
 - SEQ ID NO: 42 CrossFab₂-Fab heavy chain (HC) including two heavy chains (HC) <VEGF> with VH-VL exchange wild type (wt) coupled to one heavy chain (HC) <Ang-2> wild type (wt) via glycineserinee-linkers

10

20

25

- SEQ ID NO: 43 CrossFab₂-Fab heavy chain (HC) including two heavy chains (HC) <VEGF> with VH-VL exchange wild type (wt) coupled to one heavy chain (HC) <Ang-2> with K147E and K231E substitutions via glycine-serine-linkers
- 5 **SEQ ID NO: 44** heavy chain (HC) <VEGF> with VH-VL exchange with K147E substitution
 - **SEQ ID NO: 45** light chain (LC) <VEGF> with VH-VL exchange with Q124K substitution
 - **SEQ ID NO: 46** heavy chain (HC) <VEGF> with VH-VL exchange with K147E, and K213E substitution

SEQ ID NO: 47 light chain (LC) <VEGF> with VH-VL exchange with E123K, and Q124K substitution

Examples

15

20

10

Materials & general methods

General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991). Amino acids of antibody chains are numbered and referred to according to the numbering systems according to Kabat (Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)) as defined above.

25 **Recombinant DNA techniques**

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

30 Gene synthesis

Desired gene segments were prepared from oligonucleotides made by chemical synthesis. The 600 - 1800 bp long gene segments, which were flanked by singular restriction endonuclease cleavage sites, were assembled by annealing and ligating

- 65 -

oligonucleotides including PCR amplification and subsequently cloned via the indicated restriction sites e.g. KpnI/ SacI or AscI/PacI into a pPCRScript (Stratagene) based pGA4 cloning vector. The DNA sequences of the subcloned gene fragments were confirmed by DNA sequencing. Gene synthesis fragments were ordered according to given specifications at Geneart (Regensburg, Germany).

5

DNA sequence determination

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

10 **DNA and protein sequence analysis and sequence data management**

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

Expression vectors

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

Beside the antibody expression cassette the vectors contained:

- an origin of replication which allows replication of this plasmid in *E. coli*, and
- a β-lactamase gene which confers ampicillin resistance in *E. coli*.

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

- 25 unique restriction site(s) at the 5' end
 - the immediate early enhancer and promoter from the human cytomegalovirus,
 - followed by the Intron A sequence in the case of the cDNA organization,
 - a 5'-untranslated region of a human antibody gene,
 - an immunoglobulin heavy chain signal sequence,
- the human antibody chain (wildtype or with domain exchange) either as cDNA or
 as genomic organization with the immunoglobulin exon-intron organization

- a 3' untranslated region with a polyadenylation signal sequence, and

- unique restriction site(s) at the 3' end.

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

Cell culture techniques

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

Multispecific antibodies were expressed by transient co-transfection of the respective expression plasmids in adherently growing HEK293-EBNA or in HEK29-F cells growing in suspension as described below.

Transient transfections in HEK293-EBNA system

Multispecific antibodies were expressed by transient co-transfection of the respective expression plasmids (e.g. encoding the heavy and modified heavy chain, as well as the corresponding light and modified light chain) in adherently growing 20 HEK293-EBNA cells (human embryonic kidney cell line 293 expressing Epstein-Barr-Virus nuclear antigen; American type culture collection deposit number ATCC # CRL-10852, Lot. 959 218) cultivated in DMEM (Dulbecco's modified Eagle's medium, Gibco®) supplemented with 10% Ultra Low IgG FCS (fetal calf serum, Gibco®), 2 mM L-Glutamine (Gibco®), and 250 µg/ml Geneticin (Gibco®). For transfection FuGENE[™] 6 Transfection Reagent (Roche Molecular 25 Biochemicals) was used in a ratio of FuGENE[™] reagent (µl) to DNA (µg) of 4:1 (ranging from 3:1 to 6:1). Proteins were expressed from the respective plasmids using a molar ratio of (modified and wildtype) light chain and heavy chain encoding plasmids of 1:1 (equimolar) ranging from 1:2 to 2:1, respectively. Cells 30 were fed at day 3 with L-Glutamine ad 4 mM, Glucose [Sigma] and NAA [Gibco®]. Multispecific antibody containing cell culture supernatants were harvested from day 5 to 11 after transfection by centrifugation and stored at -20°C. the recombinant expression of human General information regarding

15

immunoglobulins in e.g. HEK293 cells is given in: Meissner, P. *et al.*, Biotechnol. Bioeng. 75 (2001) 197-203.

Transient transfections in HEK293-F system

Multispecific antibodies were generated by transient transfection with the 5 respective plasmids (e.g. encoding the heavy and modified heavy chain, as well as the corresponding light and modified light chain) using the HEK293-F system (Invitrogen) according to the manufacturer's instruction. Briefly, HEK293-F cells (Invitrogen) growing in suspension either in a shake flask or in a stirred fermenter in serum-free FreeStyle[™] 293 expression medium (Invitrogen) were transfected 10 with a mix of the four expression plasmids and 293 fectin[™] or fectin (Invitrogen). For 2 L shake flask (Corning) HEK293-F cells were seeded at a density of 1.0E*6 cells/mL in 600 mL and incubated at 120 rpm, 8% CO2. The day after the cells were transfected at a cell density of ca. 1.5E*6 cells/mL with ca. 42 mL mix of A) 20 mL Opti-MEM (Invitrogen) with 600 µg total plasmid DNA (1 µg/mL) 15 encoding the heavy or modified heavy chain, respectively and the corresponding light chain in an equimolar ratio and B) 20 ml Opti-MEM + 1.2 mL 293 fectin or fectin (2 µl/mL). According to the glucose consumption glucose solution was added during the course of the fermentation. The supernatant containing the secreted antibody was harvested after 5-10 days and antibodies were either directly 20 purified from the supernatant or the supernatant was frozen and stored.

Protein determination

The protein concentration of purified antibodies and derivatives was determined by determining the optical density (OD) at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence according to Pace, *et al.*, Protein Science, 1995, 4, 2411-1423.

Antibody concentration determination in supernatants

The concentration of antibodies and derivatives in cell culture supernatants was estimated by immunoprecipitation with Protein A Agarose-beads (Roche). 60 μ L Protein A Agarose beads were washed three times in TBS-NP40 (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Nonidet-P40). Subsequently, 1 -15 mL cell culture supernatant were applied to the Protein A Agarose beads pre-equilibrated in TBS-NP40. After incubation for at 1 hour at room temperature the beads were washed on an Ultrafree-MC-filter column (Amicon) once with 0.5 mL TBS-NP40, twice

30

25

with 0.5 mL 2x phosphate buffered saline (2xPBS, Roche) and briefly four times with 0.5 mL 100 mM Na-citrate pH 5.0. Bound antibody was eluted by addition of 35 µl NuPAGE® LDS Sample Buffer (Invitrogen). Half of the sample was combined with NuPAGE® Sample Reducing Agent or left unreduced, respectively, and heated for 10 min at 70°C. Consequently, 5-30 ul were applied to a 4-12% NuPAGE® Bis-Tris SDS-PAGE (Invitrogen) (with MOPS buffer for non-reduced SDS-PAGE and MES buffer with NuPAGE® Antioxidant running buffer additive (Invitrogen) for reduced SDS-PAGE) and stained with Coomassie Blue.

The concentration of antibodies and derivatives in cell culture supernatants was 10 quantitatively measured by affinity HPLC chromatography. Briefly, cell culture supernatants containing antibodies and derivatives that bind to Protein A were applied to an Applied Biosystems Poros A/20 column in 200 mM KH2PO4, 100 mM sodium citrate, pH 7.4 and eluted from the matrix with 200 mM NaCl, 100 mM citric acid, pH 2,5 on an Agilent HPLC 1100 system. The eluted protein was 15 quantified by UV absorbance and integration of peak areas. A purified standard IgG1 antibody served as a standard.

Alternatively, the concentration of antibodies and derivatives in cell culture supernatants was measured by Sandwich-IgG-ELISA. Briefly, StreptaWell High Bind Strepatavidin A-96 well microtiter plates (Roche) are coated with 100 µL/well biotinylated anti-human IgG capture molecule F(ab')2<h-Fcy> BI 20 (Dianova) at 0.1 µg/mL for 1 hour at room temperature or alternatively overnight at 4°C and subsequently washed three times with 200 µL/well PBS, 0.05% Tween (PBST, Sigma). 100 µL/well of a dilution series in PBS (Sigma) of the respective antibody containing cell culture supernatants was added to the wells and incubated for 1-2 hour on a microtiterplate shaker at room temperature. The wells were washed three times with 200 µL/well PBST and bound antibody was detected with 100 µl F(ab')2<hFcy>POD (Dianova) at 0.1 µg/mL as the detection antibody for 1-2 hours on a microtiterplate shaker at room temperature. Unbound detection antibody was washed away three times with 200 µL/well PBST and the bound 30 detection antibody was detected by addition of 100 µL ABTS/well. Determination of absorbance was performed on a Tecan Fluor Spectrometer at a measurement wavelength of 405 nm (reference wavelength 492 nm).

10

15

Protein purification

Proteins were purified from filtered cell culture supernatants referring to standard protocols. In brief, antibodies were applied to a Protein A Sepharose column (GE healthcare) and washed with PBS. Elution of antibodies was achieved at pH 2.8 followed by immediate neutralization of the sample. Aggregated protein was separated from monomeric antibodies by size exclusion chromatography (Superdex 200, GE Healthcare) in PBS or in 20 mM Histidine, 150 mM NaCl pH 6.0. Monomeric antibody fractions were pooled, concentrated (if required) using e.g., a MILLIPORE Amicon Ultra (30 MWCO) centrifugal concentrator, frozen and stored at -20°C or -80°C. Part of the samples were provided for subsequent protein analytics and analytical characterization e.g. by SDS-PAGE, size exclusion chromatography (SEC) or mass spectrometry.

SDS-PAGE

The NuPAGE® Pre-Cast gel system (Invitrogen) was used according to the manufacturer's instruction. In particular, 10% or 4-12% NuPAGE® Novex® Bis-TRIS Pre-Cast gels (pH 6.4) and a NuPAGE® MES (reduced gels, with NuPAGE® Antioxidant running buffer additive) or MOPS (non-reduced gels) running buffer was used.

Analytical size exclusion chromatography

Size exclusion chromatography (SEC) for the determination of the aggregation and oligomeric state of antibodies was performed by HPLC chromatography. Briefly, Protein A purified antibodies were applied to a Tosoh TSKgel G3000SW column in 300 mM NaCl, 50 mM KH₂PO₄/K₂HPO₄, pH 7.5 on an Agilent HPLC 1100 system or to a Superdex 200 column (GE Healthcare) in 2 x PBS on a Dionex HPLC-System. The eluted protein was quantified by UV absorbance and integration of peak areas. BioRad Gel Filtration Standard 151–1901 served as a standard.

Mass spectrometry

30

This section describes the characterization of the multispecific antibodies with VH/VL exchange (VH/VL CrossMabs) with emphasis on their correct assembly.
The expected primary structures were analyzed by electrospray ionization mass spectrometry (ESI-MS) of the deglycosylated intact CrossMabs and

deglycosylated/plasmin digested or alternatively deglycosylated/limited LysC digested CrossMabs.

The VH/VL CrossMabs were deglycosylated with N-Glycosidase F in a phosphate or Tris buffer at 37°C for up to 17 h at a protein concentration of 1 mg/ml. The plasmin or limited LysC (Roche) digestions were performed with 100 μg deglycosylated VH/VL CrossMabs in a Tris buffer pH 8 at room temperature for 120 hours and at 37°C for 40 min, respectively. Prior to mass spectrometry the samples were 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).

Determination of binding and binding affinity of multispecific antibodies to the respective antigens using surface plasmon resonance (SPR) (BIACORE)

Binding of the generated antibodies to the respective antigens (e.g ANG2 and VEGF) is investigated by surface plasmon resonance using a BIACORE instrument

15 (GE Healthcare Biosciences AB, Uppsala, Sweden). Briefly, for affinity 109-005-098 measurements Goat-Anti-Human IgG, JIR antibodies are immobilized on a CM5 chip via amine coupling for presentation of the antibodies against the respective antigen. Binding is measured in HBS buffer (HBS-P (10 mM HEPES, 150 mM NaCl, 0.005% Tween 20, ph 7.4), 25°C (or alternatively at 37°C) Antigen (R&D Systems or in house purified) was added in various 20 concentrations in solution. Association was measured by an antigen injection of 80 seconds to 3 minutes; dissociation was measured by washing the chip surface with HBS buffer for 3 - 10 minutes and a KD value was estimated using a 1:1 Langmuir binding model. Negative control data (e.g. buffer curves) are subtracted from 25 sample curves for correction of system intrinsic baseline drift and for noise signal reduction. The respective Biacore Evaluation Software is used for analysis of sensorgrams and for calculation of affinity data.

5

Example 1A

Production and expression of multispecific antibodies which bind to Angiopoietin-2 (ANG2) and VEGF with VH/VL domain exchange/replacement (*CrossMAb*^{Vh-VL}) in one binding arm and with single charged amino acid substitutions in the CH1/CL interface

In a first example multispecific antibodies which binds to human Angiopoietin-2 (ANG2) and human VEGF were generated as described in the general methods section by classical molecular biology techniques and is expressed transiently in HEK293 cells as described above. A general scheme of these respective multispecific, antibodies is given in Figures 1A to C. For comparison also the wild type (wt) VH/VL domain exchange/replacement antibodies with no substitution in the CH1/CL interface was prepared. Also other alternative substitutions in close proximity in the CH1CL interface (mentioned e.g. in EP 2647707) were used for comparison. The multispecific antibodies were expressed using expression plasmids containing the nucleic acids encoding the amino acid sequences depicted in Table 2a.

10

15

<u>Table 2a:</u> Amino acid sequences of light chains (LC) and heavy chains (HC) of anti-*Ang2-VEGF* multispecific antibodies Ang2VEGF-0273, Ang2VEGF-0396,Ang2VEGF-0397, Ang2VEGF-0394, Ang2VEGF-0395 with VH/VL domain exchange/replacement (*CrossMAb*^{Vh-VL}): wild type (wt) and different combinations of single charged amino acids substitutions

Antibody	LC ANG-2	HC ANG-2	HC VEGF	LC VEGF
Ang2VEGF- 0273	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF- 0396	SEQ ID NO: 5	SEQ ID NO: 6	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF- 0397	SEQ ID NO: 5	SEQ ID NO: 7	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF- 0394	SEQ ID NO: 8	SEQ ID NO: 6	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF- 0395	SEQ ID NO: 8	SEQ ID NO: 7	SEQ ID NO: 3	SEQ ID NO: 4

For all constructs knobs into holes heterodimerization technology was used with a typical knob (T366W) substitution in the first CH3 domain and the corresponding hole substitutions (T366S, L368A and Y407V) in the second CH3 domain (as well as two additional introduced cysteine residues S354C/Y349'C) (contained in the respective corresponding heavy chain (HC) sequences depicted above)

Example 1B

Purification and characterization of multispecific antibodies which bind to Angiopoietin-2 (ANG2) and VEGF with VH/VL domain

exchange/replacement (*CrossMAb^{Vh-VL}*) in one binding arm and with single charged amino acid substitutions in the CH1/CL interface

The multispecific antibodies expressed above were purified from the supernatant by a combination of Protein A affinity chromatography and size exclusion chromatography. All multispecific antibodies can be produced in good yields and are stable.

10

5

The obtained products were characterized for identity by mass spectrometry and analytical properties such as purity by SDS-PAGE, monomer content and stability

Mass spectrometry

5

10

15

The expected primary structures were analyzed by electrospray ionization mass spectrometry (ESI-MS) of the deglycosylated intact CrossMabs and deglycosylated/plasmin digested or alternatively deglycosylated/limited LysC digested CrossMabs.

The VH/VL CrossMabs were deglycosylated with N-Glycosidase F in a phosphate or Tris buffer at 37°C for up to 17 h at a protein concentration of 1 mg/ml. The plasmin or limited LysC (Roche) digestions were performed with 100 µg deglycosylated VH/VL CrossMabs in a Tris buffer pH 8 at room temperature for 120 hours and at 37°C for 40 min, respectively. Prior to mass spectrometry the samples were 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).

Results are shown in Table 2b and Figure 4a.

- 74 -

	CL ANG-2 (position 124)	CL ANG-2 (position 123)	CH1 ANG-2 (position 147)	CH1 ANG-2 (position 213)	CH1 VEGF	CL VEGF	Main side product (Bence- Jones type mispairing) % by MS
Ang2VEGF -0273	wt: Q124	wt: E123	wt: K147	wt: K213	wt	wt	<u>~20</u>
Ang2VEGF -0396	Q124K	wt	K147E	wt	wt	wt	<u>~3</u>
Ang2VEGF -0397	Q124K	wt	wt	K213E	wt	wt	<u>~3</u>
Ang2VEGF -0394	wt	E123K	K147E	wt	wt	wt	~15
Ang2VEGF -0395	wt	E123K	wt	K213E	wt	wt	~15

Table 2b: Reduction of main Bence-Jones-type side product by single charged	
amino acids substitutions according to the invention in the CH1/CL interface	

5

10

Results in Table 2b and Figure 4a show that with the substitutions of single charged amino acids with the opposite charge in the CH1 and CL domains according to the invention/as described for the invention (CL:Q124K and CH1:K147E pair; or CL:Q124K and CH1:K213E pair) the main side product (Bence-Jones type mispairing) is strongly reduced when compared to the wild type multispecific antibody without such substitutions ($\sim 17\%$ reduction). With other substitutions in close proximity (CL:Q123K and CH1:K147E pair; or CL:Q123K and CH1:K147E pair; or CL:Q123K and CH1:K213E pair) only a slight reduction of the main side product compared to the wild type multispecific antibody without such substitutions ($\sim 5\%$ reduction).

Example 1C

15

Antigen binding properties of multispecific antibodies which bind to Angiopoietin-2 (ANG2) and VEGF with VH/VL domain exchange/replacement ($CrossMAb^{Vh-VL}$) in one binding arm and with single charged amino acid substitutions in the CH1/CL interface

Binding of the multispecific antibodies of the previous examples 1A and 1B to their respective target antigens, i.e. ANG2 and VEGF, was assessed by Biacore®.

- 75 -

VEGF binding was assessed according to the following procedure:

Binding of indicated antibodies to human VEGFA-121 was investigated by surface plasmon resonance using a BIACORE® T200 instrument (GE Healthcare). Around 10000 (RU) of anti His antibody (1 µg/ml anti His antibody; Order Code: 28995056: GE Healthcare Bio-Sciences AB, Sweden) were coupled on a Series S CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. HBS-N (10 mM HEPES, 150 mM NaCl pH 7.4, GE Healthcare) was used as running buffer during the immobilization procedure. For the following kinetic characterization, sample and running buffer was PBS-T (10 mM phosphate buffered saline including 0.05% Tween20) at 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 prior to kinetic characterization.

VEFGA-121-His was captured by injecting a 0.5 µg/ml solution for 30 sec at a flow of 5 ul/min. The association was measured by injection of the indicated antibodies in various concentrations in solution for 180 sec at a flow of 30 µl/min starting with 1000 nM in 1:3 serial dilutions. The dissociation phase was monitored for up to 600 sec and triggered by switching from the sample solution to running buffer. The surface was regenerated by 60 sec washing with a Glycine pH 1.5 solution at a flow rate of 30 µl/min. Bulk refractive index differences were 20 corrected by subtracting the response obtained from a anti His antibody surface. Blank injections are also subtracted (= double referencing). For calculation of K_D and other kinetic parameters the Langmuir 1:1 model was used.

Ang-2 binding was assessed according to the following procedure:

Binding of indicated antibodies to human Ang-2-RBD-Fc was investigated by 25 surface plasmon resonance using a BIACORE® T200 instrument (GE Healthcare). Around 8000 (RU) of goat anti human F(ab')₂ (10 µg/ml anti human F(ab)'₂; Order Code: 28958325; GE Healthcare Bio-Sciences AB, Sweden) were coupled on a Series S CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. HBS-N (10 mM HEPES, 150 mM 30 NaCl pH 7.4, GE Healthcare) was used as running buffer during the immobilization procedure. For the following kinetic characterization, sample and running buffer was PBS-T (10 mM phosphate buffered saline including 0.05%

15

5

Tween20) at pH 7.4. The flow cell was set to 25 $^{\circ}$ C - and the sample block set to 12 $^{\circ}$ C - and primed with running buffer twice prior to kinetic characterization.

The bispecific antibody was captured by injecting a 5 nM solution for 25 sec at a flow of 5 μ l/min. The association was measured by injection of human Ang2-RBD-Fc in various concentrations in solution for 120 sec at a flow of 30 μ l/min starting with 100 nM in 1:3 serial dilutions. The dissociation phase was monitored for up to 180 sec and triggered by switching from the sample solution to running buffer. The surface was regenerated by 60 sec washing with a Glycine pH 2.1 solution at a flow rate of 30 μ l/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 the Langmuir 1:1 model was used.

As comparative example, a reference antibody specifically binding to Ang2 and VEGF comprising a VH/VL domain exchange/replacement but lacking charged amino acid substitutions (Ang2VEGF-0273 antibody of **Table 2b**) was assessed in parallel.

Results are indicated in Tables 2c and 2d.

Sample	KD (nM)
Ang2VEGF-0273	6
Ang2VEGF-0396	3
Ang2VEGF-0397	4
Ang2VEGF-0394	3
Ang2VEGF-0395	4

Table 2c: Affinity for VEGF of indicated antibodies

10

- 77 -

Sample	KD (nM)
Ang2VEGF-0273	15
Ang2VEGF-0396	17
Ang2VEGF-0397	14
Ang2VEGF-0394	12
Ang2VEGF-0395	15

<u>Table 2d:</u> Affinity for Ang2 of indicated antibodies

All tested antibodies specifically bind to both targets, Ang2 and VEGF, and exhibit an antigen affinity in the nanomolar range.

Example 1D

5 Stability of multispecific antibodies which bind to Angiopoietin-2 (ANG2) and VEGF with VH/VL domain exchange/replacement (*CrossMAb*^{Vh-VL}) in one binding arm and with single charged amino acid substitutions in the CH1/CL interface

In order to assess stability of the antibody constructs, thermal stability as well as aggregation onset temperatures were assessed according to the following procedure.

Samples of the indicated antibodies were prepared at a concentration of 1 mg/mL in 20 mM Histidine/Histidine chloride, 140 mM NaCl, pH 6.0, transferred into a 10 μ L micro-cuvette array and static light scattering data as well as fluorescence data upon excitation with a 266 nm laser were recorded with an Optim1000 instrument (Avacta Inc.), while the samples were heated at a rate of 0.1 °C/min from 25°C to 90°C.

The aggregation onset temperature (T_{agg}) is defined as the temperature at which the scattered light intensity starts to increase. The melting temperature (T_m) is defined as the inflection point in a fluorescence intensity vs. wavelength graph.

Results are shown in Table 2e.

15

Sample	T _{agg} (°C)	T _m (°C)
Ang2VEGF-0273	56,0	61,3
Ang2VEGF-0396	56,9	62,0
Ang2VEGF-0397	56,0	61,7
Ang2VEGF-0394	56,9	62,2
Ang2VEGF-0395	56,8	62,1

<u>Table 2e:</u> Aggregation onset temperature (T_{agg}) and melting temperature (T_m) of indicated antibodies

5 Example 1E

Production yield of multispecific antibodies which bind to Angiopoietin-2 (ANG2) and VEGF with VH/VL domain exchange/replacement ($CrossMAb^{Vh-}V^L$) in one binding arm and with single charged amino acid substitutions in the CH1/CL interface

10 Production yields of the indicated multispecific antibodies were assessed after Protein A purification (ProtA). Results are shown in **Table 2f**.

Table 2e: Production yields [mg/L supernatant] of indicated antibodies

Sample	ProtA
Ang2VEGF-0273	65
Ang2VEGF-0396	80.8
Ang2VEGF-0397	68.4
Ang2VEGF-0394	79.2
Ang2VEGF-0395	93.6

Example 2A

5

10

15

Production and expression of multispecific antibodies which bind to Angiopoietin-2 (ANG2) and VEGF with VH/VL domain exchange/replacement (*CrossMAb*^{Vh-VL}) in one binding arm and with different charged amino acid substitutions in the CH1/CL interface

In a first example multispecific antibodies which binds to human Angiopoietin-2 (ANG2) and human VEGF were generated as described in the general methods section by classical molecular biology techniques and is expressed transiently in HEK293 cells as described above. A general scheme of these respective multispecific, antibodies is given in Figures 1A to C. For comparison also the wild type (wt) VH/VL domain exchange/replacement antibodies with no substitution in the CH1/CL interface was prepared. The multispecific antibodies were expressed using expression plasmids containing the nucleic acids encoding the amino acid sequences depicted in Table 3a.

<u>Table 3a:</u> Amino acid sequences of light chains (LC) and heavy chains (HC) of anti-*Ang2-VEGF* multispecific antibodies Ang2VEGF-0273, Ang2VEGF-0274, Ang2VEGF-0282, Ang2VEGF-0283, Ang2VEGF-0284, Ang2VEGF-0285, Ang2VEGF-0286 with VH/VL domain exchange/replacement (*CrossMAb*^{Vh-VL}): wild type (wt) and different combinations of charged amino acids substitutions

5

Antibody	LC ANG-2	HC ANG-2	HC VEGF	LC VEGF
Ang2VEGF-0273	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0274	SEQ ID NO: 9	SEQ ID NO: 10	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0282	SEQ ID NO: 11	SEQ ID NO: 10	SEQ ID NO: 3	SEQ ID NO: 12
Ang2VEGF-0283	SEQ ID NO: 13	SEQ ID NO: 10	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0284	SEQ ID NO: 11	SEQ ID NO: 14	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0285	SEQ ID NO: 11	SEQ ID NO: 14	SEQ ID NO: 3	SEQ ID NO: 12
Ang2VEGF-0286	SEQ ID NO: 9	SEQ ID NO: 10	SEQ ID NO: 3	SEQ ID NO: 12

For all constructs knobs into holes heterodimerization technology was used with a typical knob (T366W) substitution in the first CH3 domain and the corresponding hole substitutions (T366S, L368A and Y407V) in the second CH3 domain (as well as two additional introduced cysteine residues S354C/Y349'C) (contained in the

respective corresponding heavy chain (HC) sequences depicted above).

Example 2B

Purification and characterization of multispecific antibodies which bind to Angiopoietin-2 (ANG2) and VEGF with VH/VL domain exchange/replacement (*CrossMAb*^{Vh-VL}) in one binding arm and with different charged amino acid substitutions in the CH1/CL interface

5

15

20

The multispecific antibodies expressed above were purified from the supernatant by a combination of Protein A affinity chromatography and size exclusion chromatography. All multispecific antibodies can be produced in good yields and are stable.

10 The obtained products were characterized for identity by mass spectrometry and analytical properties such as purity by SDS-PAGE, monomer content and stability

Mass spectrometry

The expected primary structures were analyzed by electrospray ionization mass spectrometry (ESI-MS) of the deglycosylated intact CrossMabs and deglycosylated/plasmin digested or alternatively deglycosylated/limited LysC digested CrossMabs.

The VH/VL CrossMabs were deglycosylated with N-Glycosidase F in a phosphate or Tris buffer at 37°C for up to 17 h at a protein concentration of 1 mg/ml. The plasmin or limited LysC (Roche) digestions were performed with 100 µg deglycosylated VH/VL CrossMabs in a Tris buffer pH 8 at room temperature for 120 hours and at 37°C for 40 min, respectively. Prior to mass spectrometry the samples were 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).

25 Results are shown in Table 3b and Figure 5a.

	CL ANG-2	CL ANG-2	CH1 ANG-2	CH1 ANG-2	CH1 VEGF	CL VEGF	Main side product (Bence- Jones type mispairing) % by MS
Ang2VEGF- 0273	wt: Q124 (kappa)	wt: E123	wt: K147	wt: K213	wt	wt: Q124	<u>~20%</u>
Ang2VEGF- 0274	Q124K	E123K	K147E	K213E	wt	wt	0
Ang2VEGF- 0282	Q124R	E123K	K147E	K213E	wt	Q124E	0
Ang2VEGF- 0283	E124K (lambda)	E123K	K147E	K213E	wt	wt:	0
Ang2VEGF- 0284	Q124R	E123K	K147E	K213D	wt	wt	0
Ang2VEGF- 0285	Q124R	E123K	K147E	K213D	wt	Q124E	0
Ang2VEGF- 0286	Q124K	E123K	K147E	K213E	wt	Q124E	0

<u>Table 3b:</u> Reduction of main Bence-Jones-type side product by single charged amino acids substitutions according to the invention in the CH1/CL interface

Results in Table 3b and Figure 5a show that with the double substitutions of charged amino acids with the opposite charge in the CH1 and CL domains according to the invention/as described for the invention (CL:Q124K/E123K and CH1:K147E/K213E; CL:Q124R/E123K and CH1:K147E/K213E; CL:Q124R/E123K and CH1:K147E/K213D) the main side product (Bence-Jones type mispairing) is <u>completely removed</u> when compared to the wild type multispecific antibody without such substitutions. This is independent of the further single substitution Q124E in the CL domain of the other binding arm, which does not influence the expression nor side product profile.

Example 2C

Antigen binding properties of multispecific antibodies which bind to Angiopoietin-2 (ANG2) and VEGF with VH/VL domain exchange/replacement ($CrossMAb^{Vh-VL}$) in one binding arm and with different charged amino acid substitutions in the CH1/CL interface

5

10

Binding of the multispecific antibodies of the previous examples 2A and 2B to their respective target antigens, i.e. ANG2 and VEGF, was assessed by Biacore® as outlined in example 1C.

As comparative example, the reference antibody specifically binding to Ang2 and VEGF comprising a VH/VL domain exchange/replacement but lacking charged amino acid substitutions (Ang2VEGF-0273 antibody of **Table 2b**) was assessed in parallel.

Results are indicated in Tables 3c and 3d.

Sample	KD (nM)
Ang2VEGF-0273	6
Ang2VEGF-0274	3
Ang2VEGF-0282	4
Ang2VEGF-0283	4
Ang2VEGF-0284	4
Ang2VEGF-0285	4
Ang2VEGF-0286	4

Table 3c: Affinity for VEGF of indicated antibodies

- 84 -

Sample	KD (nM)
Ang2VEGF-0273	15
Ang2VEGF-0274	17
Ang2VEGF-0282	14
Ang2VEGF-0283	15
Ang2VEGF-0284	13
Ang2VEGF-0285	14
Ang2VEGF-0286	12

Table 3d: Affinity for Ang2 of indicated antibodies

All tested antibodies specifically bind to both targets, Ang2 and VEGF, and exhibit an antigen affinity in the nanomolar range.

5 <u>Example 2D</u>

Stability of multispecific antibodies which bind to Angiopoietin-2 (ANG2) and VEGF with VH/VL domain exchange/replacement ($CrossMAb^{Vh-VL}$) in one binding arm and with single charged amino acid substitutions in the CH1/CL interface

10 In order to assess stability of the antibody constructs, thermal stability as well as aggregation onset temperatures were assessed as outlined in example 1D.

Results are shown in Table 3e.

Sample	T _{agg} (°C)	T _m (°C)
Ang2VEGF-0273	56,0	61,3
Ang2VEGF-0274	53,5	58,9
Ang2VEGF-0282	56,9	61,4
Ang2VEGF-0283	56,3	61,0
Ang2VEGF-0284	56,3	61,1
Ang2VEGF-0285	56,3	61,1
Ang2VEGF-0286	56,3	61,6

<u>Table 3e:</u> Aggregation onset temperature (T_{agg}) and melting temperature (T_m) of indicated antibodies

Example 3A

5 Production and expression of multispecific antibodies which bind to IL-17 and TWEAK with VH/VL domain exchange/replacement (*CrossMAb*^{Vh-VL}) in one binding arm and with different charged amino acid substitutions in the CH1/CL interface

10

In a first example multispecific antibodies which binds to human IL-17 and human TWEAK were generated as described in the general methods section by classical molecular biology techniques and expressed transiently in HEK293 cells as described above. A general scheme of these respective multispecific, antibodies is given in Figures 1A to C. For comparison also the wild type (wt) VH/VL domain exchange/replacement antibodies with no substitution in the CH1/CL interface was prepared. The multispecific antibodies were expressed using expression plasmids containing the nucleic acids encoding the amino acid sequences depicted in Table 4a.

<u>Table 4a:</u> Amino acid sequences of light chains (LC) and heavy chains (HC) of anti-*TWEAK-IL17* multispecific antibodies TweakIL17-0096, TweakIL17-0097, TweakIL17-0098, TweakIL17-0099, TweakIL17-0100, TweakIL17-0101 with VH/VL domain exchange/replacement (*CrossMAb*^{Vh-VL}): wild type (wt) and different combinations of charged amino acids substitutions

	,	

Antibody	LC IL17	HC IL17	HC TWEAK	LC TWEAK
TweakIL17-0096	SEQ ID NO: 15	SEQ ID NO: 16	SEQ ID NO: 17	SEQ ID NO: 18
TweakIL17-0097	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 17	SEQ ID NO: 21
TweakIL17-0098	SEQ ID NO: 19	SEQ ID NO: 22	SEQ ID NO: 17	SEQ ID NO: 18
TweakIL17-0099	SEQ ID NO: 19	SEQ ID NO: 22	SEQ ID NO: 17	SEQ ID NO: 21
TweakIL17-0100	SEQ ID NO: 23	SEQ ID NO: 20	SEQ ID NO: 17	SEQ ID NO: 21
TweakIL17-0101	SEQ ID NO: 23	SEQ ID NO: 20	SEQ ID NO: 17	SEQ ID NO: 18

For all constructs knobs into holes heterodimerization technology was used with a typical knob (T366W) substitution in the first CH3 domain and the corresponding hole substitutions (T366S, L368A and Y407V) in the second CH3 domain (as well as two additional introduced cysteine residues S354C/Y349'C) (contained in the respective corresponding heavy chain (HC) sequences depicted above).

Example 3B

15

10

Purification and characterization of multispecific antibodies which bind to IL-17 and TWEAK with VH/VL domain exchange/replacement (*CrossMAb*^{Vh-VL}) in one binding arm and with different charged amino acid substitutions in the CH1/CL interface

The multispecific antibodies expressed above were purified from the supernatant by a combination of Protein A affinity chromatography and size exclusion chromatography. All multispecific antibodies can be produced in good yields and are stable.

The obtained products were characterized for identity by mass spectrometry and analytical properties such as purity by SDS-PAGE, monomer content and stability

Mass spectrometry

The expected primary structures were analyzed by electrospray ionization mass spectrometry (ESI-MS) of the deglycosylated intact CrossMabs and deglycosylated/plasmin digested or alternatively deglycosylated/limited LysC digested CrossMabs.

The VH/VL CrossMabs were deglycosylated with N-Glycosidase F in a phosphate or Tris buffer at 37°C for up to 17 h at a protein concentration of 1 mg/ml. The plasmin or limited LysC (Roche) digestions were performed with 100 µg deglycosylated VH/VL CrossMabs in a Tris buffer pH 8 at room temperature for 120 hours and at 37°C for 40 min, respectively. Prior to mass spectrometry the samples were 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).

Results are shown in Table 4b and Figure 6a.

15 <u>**Table 4b:**</u> Reduction of main Bence-Jones-type side product by single charged amino acids substitutions according to the invention in the CH1/CL interface

	CL IL17 (positio n 124)	CL IL17 (position 123)	CH1 IL17 (position 147)	CH1 IL17 (positi on 213)	CH1 TWEAK	CL TWEAK (position 124)	Main side product (Bence- Jones type mispairing) % by MS
TweakIL17- 0096	wt: Q124	wt: E123	wt: K147	wt: K213	wt	wt: Q124	<u>~20%</u>
TweakIL17- 0097	Q124K	E123R	K147E	K213E	wt	Q124E	0
TweakIL17- 0098	Q124K	E123R	K147E	K213D	wt	wt	0
TweakIL17- 0099	Q124K	E123R	K147E	K213D	wt	Q124E	0
TweakIL17- 0100	Q124K	E123K	K147E	K213E	wt	Q124E	0
TweakIL17- 0101	Q124K	E123K	K147E	K213E	wt	wt	not determ.

10

Results in Table 2b and Figure 6a show that with the double substitutions of charged amino acids with the opposite charge in the CH1 and CL domains according to the invention/as described for the invention (CL:Q124K/E123R and CH1:K147E/K213E; CL:Q124K/E123R and CH1:K147E/K213D; CL:Q124K/E123K and CH1:K147E/K213E) the main side product (Bence-Jones type mispairing) is <u>completely removed</u> when compared to the wild type multispecific antibody without such substitutions. This is independent of the further single substitution Q124E in the CL domain of the other binding arm, which does not influence the expression nor side product profile.

Example 4A

Production and expression of bivalent and trivalent multispecific antibodies which bind to Ang2 and VEGF, wherein the antibodies are devoid of an Fc fragments and include a VH/VL domain exchange/replacement in one binding arm and one or more charged amino acid substitutions in the CH1/CL interface

In a further example multispecific antibodies which bind to human Ang2 and human VEGF were generated as described in the general methods section by classical molecular biology techniques and expressed transiently in HEK293 cells as described above. The generated antibodies included in the binding arm specifically binding to VEGF a Fab fragment with a VH/VL domain exchange and in another binding arm specifically binding to Ang2 a Fab fragment without domain exchanges, while the multispecific antibody is devoid of an Fc fragment. Accordingly, the first light chain is derived from an antibody specifically binding to human Ang2 and comprises from N-terminal to C-terminal direction the domains VL-CL. The heavy chains of the first (anti-Ang2) and the second (anti-VEGF) antibody are connected via a glycin-serin peptide linker. In the heavy chain of the antibody specifically binding to VEGF the original variable domain VH is replaced by the variable domain VL derived from the anti-VEGF antibody. Thus, the polypeptide comprising the heavy chains of the anti-Ang2 and anti-VEGF antibodies comprises from N-terminal to C-terminal direction the domains VH(Ang2)-CH1(Ang2)-linker-VL(VEGF)-CH1(VEGF). In the light chain specifically binding to human VEGF, the original variable domain VL is replaced by the variable domain VH derived from the anti-VEGF antibody. Thus, the

20

25

30

15

5

modified light chain of the anti-VEGF antibody comprises from N-terminal to C-terminal direction the domains VH-CL. Substitutions of the distinct amino acids in the CH1/CL interface are indicated in **Table 5b**.

In this example, multispecific antibodies of three general structures were generated:

- i) bivalent multispecific Ang2-VEGF bispecific antibody of a CrossFabV_H-V_L-(Fab) format (general structure indicated Fig. 7D);
- trivalent multispecific Ang2-VEGF bispecific antibody of a (CrossFabV_H-V_L)₂-Fab format (general structure indicated in Fig. 8C (neu));
- 10 iii) trivalent multispecific Ang2-VEGF bispecific antibody of a $(Fab)_2$ -CrossFabV_H-V_L format (general structure indicated in Fig. 8D);

For comparison also the wild type (wt) VH/VL domain exchange/replacement antibodies with no substitution in the CH1/CL interface are prepared. The multispecific antibodies are expressed using expression plasmids containing the nucleic acids encoding the amino acid sequences depicted in Table 5a.

15

<u>Table 5a:</u> Amino acid sequences of light chains (LC) and heavy chains (HC) of anti-Ang2-VEGF multispecific antibodies with VH/VL domain

exchange/replacement: wild type ("uncharged") and different combinations of charged amino acids substitutions ("charged")

Antibody	LC Ang2	НС	LC VEGF
xFab-Fab <ang2-< td=""><td></td><td></td><td></td></ang2-<>			
VEGF>-uncharged	SEQ ID NO: 1	SEQ ID NO: 40	SEQ ID NO: 4
(Ang2VEGF-0452)			
xFab-Fab <ang2-< td=""><td></td><td></td><td></td></ang2-<>			
VEGF>-charged	SEQ ID NO: 11	SEQ ID NO: 41	SEQ ID NO: 4
(Ang2VEGF-0447)			
xFab ₂ -Fab <ang2-< td=""><td></td><td></td><td></td></ang2-<>			
	SEQ ID NO: 1	SEQ ID NO: 42	SEQ ID NO: 4
$\frac{(\text{Ang2VEGF-0453})}{\text{F-F-h}}$			
xFab ₂ -Fab <ang2- VEGF>-charged</ang2- 	SEQ ID NO: 11	SEQ ID NO: 43	SEQ ID NO: 4
(Ang2VEGF-0448)			`
Fab2-xFab <ang2-< td=""><td>SEO ID NO. 1</td><td>SEO ID NO. 29</td><td></td></ang2-<>	SEO ID NO. 1	SEO ID NO. 29	
VEGF>-uncharged	SEQ ID NO: 1	SEQ ID NO: 38	SEQ ID NO: 4
Fab2-xFab <ang2- VEGF>-charged</ang2- 	SEQ ID NO: 11	SEQ ID NO: 39	SEQ ID NO: 4

- 91 -

		Ang	V	EGF		
	CL (position 124)	(position (position (position		CH1	CL (position 124)	
xFab- Fab <ang2- VEGF>- uncharged (Ang2VEGF- 0452)</ang2- 	wt: Q124	wt: E123	wt: K147	wt: K213	wt	wt: Q124
xFab- Fab <ang2- VEGF>-charged (Ang2VEGF- 0447)</ang2- 	Q124R	E123K	K147E	K123E	wt	wt
xFab ₂ - Fab <ang2- VEGF>- uncharged (Ang2VEGF- 0453)</ang2- 	wt: Q124	wt: E123	wt: K147	wt: K213	wt	wt: Q124
xFab ₂ - Fab <ang2- VEGF>-charged (Ang2VEGF- 0448)</ang2- 	Q124R	E123K	K147E	K123E	wt	wt
Fab2- xFab <ang2- VEGF>- uncharged</ang2- 	wt: Q124	wt: E123	wt: K147	wt: K213	wt	wt: Q124
Fab2- xFab <ang2- VEGF>-charged</ang2- 	Q124R	E123K	K147E	K123E	wt	wt

<u>Table 5b</u>: Amino acid substitutions in the CH1/CL interface in antibodies according to the invention mentioned in Table 5a

Example 4B:

Production and expression of bivalent and trivalent multispecific antibodies which bind to ANG2 and VEGF, wherein the antibodies are devoid of an Fc fragments and include a VH/VL domain exchange/replacement in one binding arm and different charged amino acid substitutions in the CH1/CL interface

5

10

The secreted protein was purified by standard procedures using affinity purification.

Production yields after affinity purification and the fraction of the antibody molecule as determined by analytical size exclusion chromatography are indicated in **Table 5c**.

Antibody	Yield [mg/L]	Fraction [%] of antibody by analytical SEC
Ang2VEGF-0452	37.8	64.1
Ang2VEGF-0447	26.7	88.5
Ang2VEGF-0453	4.2	88.5
Ang2VEGF-0448	9.7	92.4

<u>Table 5c</u>: Production yield and desired antibody fraction after affinity purification

Mass spectrometry: The expected primary structures were analyzed by

15 electrospray ionization mass spectrometry (ESI-MS) of the deglycosylated intact antibodies and deglycosylated/plasmin digested or alternatively deglycosylated/limited LysC digested antibodies .

20

The VH/VL Fab-CrossFab constructs were deglycosylated with N-Glycosidase F in a phosphate or Tris buffer at 37°C for up to 17 h at a protein concentration of 1 mg/ml. The plasmin or limited LysC (Roche) digestions were performed with 100 µg deglycosylated VH/VL Fab-CrossFabs in a Tris buffer pH 8 at room temperature for 120 hours and at 37°C for 40 min, respectively. Prior to mass spectrometry the samples were 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).

5

Due to a overlapping mass-range between the provided material and our MS-Methods the samples were aquired with two different methods to see potential sideproducts in a bigger mass range. While working in the larger mass range (1000-4000 m/z) the method includes CID voltage (in this case a cCID of 90), the measurement in the lower mass range (600-2000) uses no CID. With the application of CID there is a higher chance to aquire fragments which appear in order to in source fragmentation in the mass spectrometer.

10 Results are shown in **Table 5d**.

<u>Table 5d</u>: Side products of indicated antibodies as analyzed by MS quantified relatively against the desired main molecule

Antibody	Fraction of side product [%] by MS	Side product
Ang2VEGF-0452	6 %	mispaired side product with two Ang2 VL-CL light chains
Ang2VEGF-0447	0	not detected
Ang2VEGF-0453	4.4 %;	mispaired side product with
		three Ang VL-CL light chains;
	35.7%	mispaired side product with two
		Ang VL-CL light chains and
		one VEGF VH-CL chain
Ang2VEGF-0448	0	not detected

Example 4C:

15 Antigen binding properties of bivalent and trivalent multispecific antibodies which bind to ANG2 and VEGF, wherein the antibodies are devoid of an Fc fragments and include a VH/VL domain exchange/replacement in one binding arm and different charged amino acid substitutions in the CH1/CL interface

Binding of the multispecific antibodies of the previous examples 4A and 4B to their respective target antigens, i.e. ANG2 and VEGF, was assessed by Biacore®.

- 94 -

VEGF binding was assessed according to the following procedure:

Binding of indicated antibodies to human VEGFA-121 was investigated by surface plasmon resonance using a BIACORE® T200 instrument (GE Healthcare). Aim for 50 RU of VEFGA-121-His were coupled on a Series S C1 chip (GE Healthcare BR-1005-35) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. HBS-N (10 mM HEPES, 150 mM NaCl pH 7.4, GE Healthcare) was used as running buffer during the immobilization procedure. For the following kinetic characterization, sample and running buffer was PBS-T (10 mM phosphate buffered saline including 0.05% Tween20) at 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 prior to kinetic characterization.

The association was measured by injection the indicated antibody in various concentrations in solution for 180 sec at a flow of 30 µl/min starting with 100 nM in 1:3 serial dilutions. The dissociation phase was monitored for up to 300 sec and triggered by switching from the sample solution to running buffer. The surface was regenerated by 30 sec washing with a 0.85% H₃PO₄ (phosphoric acid) solution at a flow rate of 30 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from a anti His antibody surface. Blank injections are also subtracted (= double referencing). For calculation of K_D and other kinetic parameters the Langmuir 1:1 model was used.

Ang-2 binding was assessed according to the following procedure:

Binding of indicated antibodies to human Ang-2-RBD-Fc was investigated by surface plasmon resonance using a BIACORE® T200 instrument (GE Healthcare). Around 8000 (RU) of goat anti human F(ab')₂ (10 µg/ml anti human F(ab)'₂; Order
Code: 28958325; GE Healthcare Bio-Sciences AB, Sweden) were coupled on a Series S CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. HBS-N (10 mM HEPES, 150 mM NaCl pH 7.4, GE Healthcare) was used as running buffer during the immobilization procedure. For the following kinetic characterization, sample and running buffer was PBS-T (10 mM phosphate buffered saline including 0.05% Tween20) at 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 prior to kinetic characterization.

15

5

10

The bispecific antibody was captured by injecting a 5 nM solution for 25 sec at a flow of 5 μ l/min. The association was measured by injection of human Ang2-RBD-Fc in various concentrations in solution for 120 sec at a flow of 30 μ l/min starting with 100 nM in 1:3 serial dilutions. The dissociation phase was monitored for up to 180 sec and triggered by switching from the sample solution to running buffer. The surface was regenerated by 60 sec washing with a Glycine pH 2.1 solution at a flow rate of 30 μ l/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.

10

5

Results are indicated in **Tables 5e and 5f**.

Table 5e: Affinity for VEGF of indicated antibodies	Table 5e:	Affinity	for	VEGF	of indicated	antibodies
--	-----------	----------	-----	------	--------------	------------

Antibody	KD (nM)
Ang2VEGF-0452	0.35
Ang2VEGF-0447	0.36
Ang2VEGF-0453	0.22
Ang2VEGF-0448	0.18

Table 5f: Affinity for Ang2 of indicated antibodies

Antibody	KD (nM)
Ang2VEGF-0452	3
Ang2VEGF-0447	3
Ang2VEGF-0453	5
Ang2VEGF-0448	4

15 Antigen binding was not impaired by the mutations introduced into the CH1/CL interface of the Fc free antibodies.

Example 5A:

Production and expression of multispecific antibodies which bind to Angiopoietin-2 (ANG2) and VEGF with VH/VL domain exchange/replacement (*CrossMAb*^{Vh-VL}) in the VEGF-binding arm and with different charged amino acid substitutions in the CH1/CL interface of the VEGF-binding arm

In a further example multispecific antibodies which bind to human Angiopoietin-2 (ANG2) and human VEGF were generated as described in the general methods section by classical molecular biology techniques and is expressed transiently in HEK293 cells as described above. A general scheme of these respective multispecific, antibodies is given in Figure 1B, indicating that the substitution with different charged amino acids is present within the CH1/CL interface of the binding arm comprising the VH/VL domain exchange/replacement. For comparison also the wild type (wt) VH/VL domain exchange/replacement antibodies with no substitution in the CH1/CL interface was prepared. The multispecific antibodies were expressed using expression plasmids containing the nucleic acids encoding the amino acid sequences depicted in Table 6a.

<u>Table 6a:</u> Amino acid sequences of light chains (LC) and heavy chains (HC) of anti-*Ang2-VEGF* multispecific antibodies Ang2VEGF-0273, Ang2VEGF-0425, and Ang2VEGF-0424 with VH/VL domain exchange/replacement (*CrossMAb*^{Vh-VL}): wild type (wt) and different combinations of charged amino acids substitutions

Antibody	LC ANG-2	HC ANG-2	HC VEGF	LC VEGF
Ang2VEGF-0273	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0425	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 44	SEQ ID NO: 45
Ang2VEGF-0424	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 46	SEQ ID NO: 47

20

5

10

For all constructs knobs into holes heterodimerization technology was used with a typical knob (T366W) substitution in the first CH3 domain and the corresponding hole substitutions (T366S, L368A and Y407V) in the second CH3 domain (as well as two additional introduced cysteine residues S354C/Y349C) (contained in the respective corresponding heavy chain (HC) sequences depicted above).

Example 5B

Purification and characterization of multispecific antibodies which bind to Angiopoietin-2 (ANG2) and VEGF with VH/VL domain

10 exchange/replacement (*CrossMAb*^{Vh-VL}) in one binding arm and with different charged amino acid substitutions in the CH1/CL interface

The multispecific antibodies expressed above were purified from the supernatant by a combination of Protein A affinity chromatography and size exclusion chromatography. All multispecific antibodies can be produced in good yields and are stable.

The obtained products were characterized for identity by mass spectrometry and analytical properties such as purity by SDS-PAGE, monomer content and stability

Mass spectrometry

The expected primary structures were analyzed by electrospray ionization mass spectrometry (ESI-MS) of the deglycosylated intact CrossMabs and deglycosylated/plasmin digested or alternatively deglycosylated/limited LysC digested CrossMabs.

The VH/VL CrossMabs were deglycosylated with N-Glycosidase F in a phosphate or Tris buffer at 37°C for up to 17 h at a protein concentration of 1 mg/ml. The plasmin or limited LysC (Roche) digestions were performed with 100 µg deglycosylated VH/VL CrossMabs in a Tris buffer pH 8 at room temperature for 120 hours and at 37°C for 40 min, respectively. Prior to mass spectrometry the samples were 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).

Results are shown in Table 6b.

20

25

30

15

<u>Table 6b:</u> Side product profile (main Bence-Jones-type side product) by single charged amino acids substitutions in the CH1/CL interface within the binding arm comprising the VH/VL domain exchange/replacement

	CL ANG-2	CH1 ANG-2	CH1 VEGF	CL VEGF	desired molecule	Main side product (Bence- Jones type mispairing) % by MS
Ang2VEGF- 0273	wt (kappa)	wt	wt K147 K213	wt E123 Q124	n.d.	~20%
Ang2VEGF- 0425	wt	wt	K147E	Q124K	72%	22%
Ang2VEGF- 0424	wt	wt	K147E K213E	E123K Q124K	64%	26 %

5 Results in Table 6b demonstrate that the side product profile (including the Bence-Jones type mispairing) could not be improved in the Ang2VEGF-bispecific antibodies with amino acid substitutions in the CH1/CL interface located within the binding arm comprising the VH/VL domain exchange/replacement.

- 99 -

Patent Claims

- 1. A multispecific antibody, comprising:
 - a) a first light chain and a first heavy chain of a first antibody which specifically binds to a first antigen; and
- b) a second light chain and a second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

10 wherein

5

15

20

25

i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index); or

ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at positions 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

- 2. A multispecific antibody, comprising:
 - a) a first light chain and a first heavy chain of a first antibody which specifically binds to a first antigen; and
 - a second light chain and a second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

5

10

15

20

25

30

wherein

in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K) or arginine (R) (numbering according to Kabat), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

3. The multispecific antibody according to claim 1 or 2,

wherein in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

4. The multispecific antibody according to claim 2,

wherein in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

5. The multispecific antibody according to claim 1,

wherein in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

6. The multispecific antibody according to claim 2,

wherein in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) (numbering according to Kabat),

and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

15 7. The antibody according to any one of the preceding claims, characterized in that

a first CH3 domain of the first heavy chain of the antibody under a) and a 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,

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

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

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

10

20

25

30

and

ii) the CH3 domain of the other heavy chain is altered,
so that within the original interface of the CH3 domain of the other
heavy chain that meets the original interface of the CH3 domain of the
one heavy chain within the multispecific antibody,
an amino acid residue is replaced with an amino acid residue having a
smaller side chain volume, thereby generating a cavity within the
interface of the CH3 domain of the other heavy chain within which a
protuberance within the interface of the CH3 domain of the one heavy

8. The antibody according to claim 7, characterized in that

15 the said amino acid residue having a larger side chain volume is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W); and said amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine (S), threonine (T) and valine (V).

20 9. The antibody according to claim 7 or 8, characterized in that

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.

- 10. A multispecific antibody according to any one of the preceding claims that specifically binds to human TWEAK and that specifically binds to human IL17, wherein
 - A) the multispecific antibody comprises
 a variable heavy chain domain (VH) of SEQ ID NO:24, and a variable
 light chain domain (VL) of SEQ ID NO:25; and
- 30 B) the multispecific antibody comprises a variable heavy chain domain (VH) of SEQ ID NO:26, and a variable light chain domain (VL) of SEQ ID NO:27.

25

5

11. A method for the preparation of a multispecific antibody according to any one of claims 1 to 10,

comprising the steps of

5

10

15

20

25

A) transforming a host cell with vectors comprising nucleic acid molecules encoding

a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and

- b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and wherein
- i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or Histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K), arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index); or
 - ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or Histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K), arginine (R)), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at positions 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index);
- 30
- B) culturing the host cell under conditions that allow synthesis of said antibody molecule; and
- C) recovering said antibody molecule from said culture.

- 12. A nucleic acid encoding the amino acid sequences of a multispecific antibody according to any one of claims 1 to 10.
- 13. An expression vector containing the nucleic acid according to claim 12 capable of expressing said nucleic acid in a host cell.
- 14. A composition comprising the antibody according to any one of claims 1 to 10.
- 15. A pharmaceutical composition comprising an antibody according to any one of claims 1 to 10 and at least one pharmaceutically acceptable excipient.

10



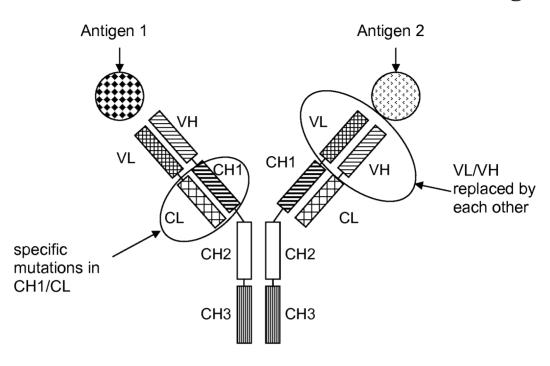
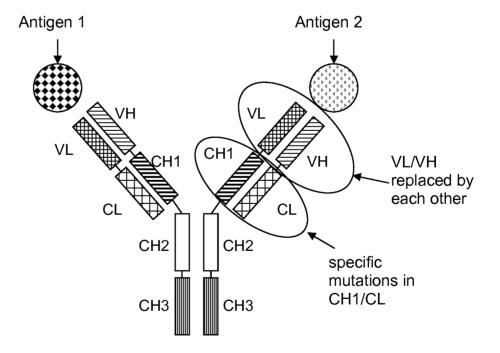
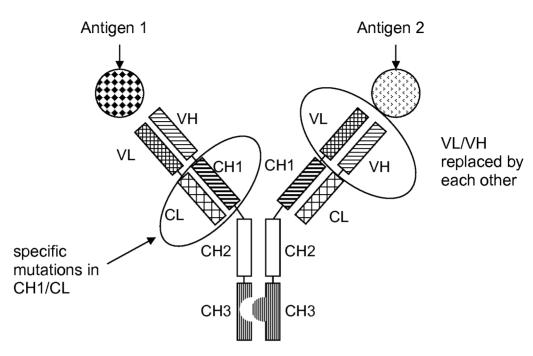
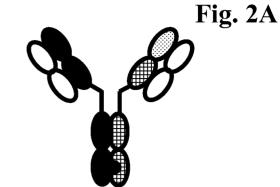


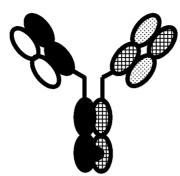
Fig. 1B











desired bispecific antibody with VH-VL exchange/replacement in one binding arm Main side product: result of Bence-Jones interaction of crossed HC with uncrossed LC

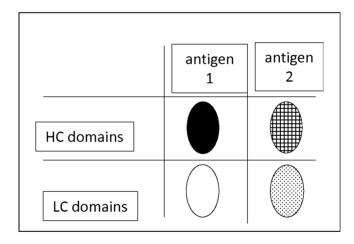
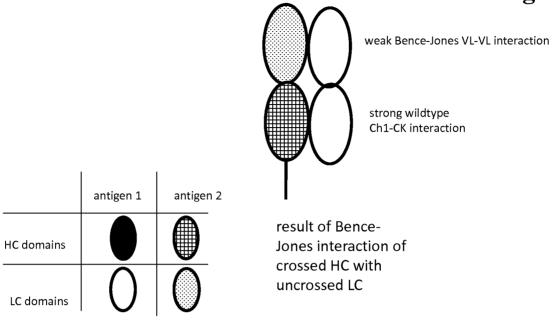


Fig. 2B



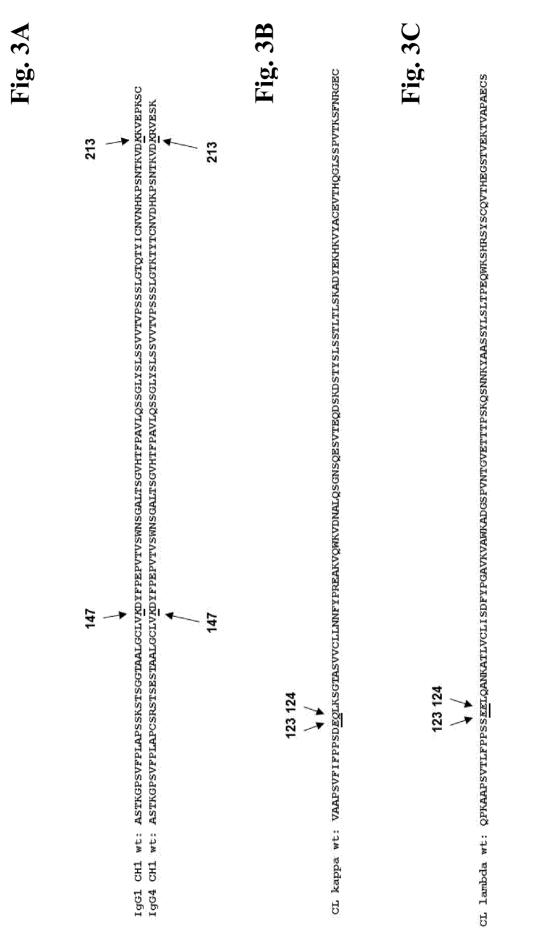
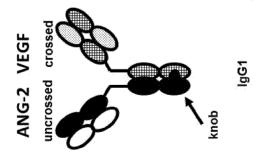


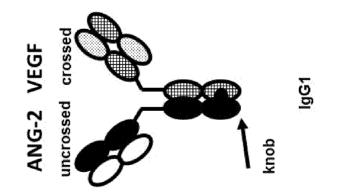
Fig. 4A

	1				-		
		Bence-Jones mispairing (VL-CH1/VL-CL- Pairing) (amount antibody with 2 Ang2-LC's) % by MS	~20	ΰı	,çi	¢15	~15
	ange – ed	CL	wt	Wť.	1M	Ŵť	wt
	VH/VL exchange crossed	CH1 VEGF	wt	wŧ	wt	wŧ.	wt
	>	CL isotype	kappa	kappa	kappa	kappa	kappa
	-	CH1 ANG-2 (position 213)	wt: K213	wt	K213E	wt	K213E
	xchange ssed	CH1 ANG-2 (position 147)	wt: K147	K147E	wt	K147E	wt
	No VH/VL exchange – uncrossed	CL ANG-2 (position 123)	wt: E123	wt	ŵt	E123K	E123K
:	No	CL ANG-2 (position 124)	wt: Q124	Q124K	Q124K	wt	wt
-		CL isotype	kappa	kappa	kappa	kappa	kappa
			Ang2VEGF -0273	Ang2VEGF -0396	Ang2VEGF -0397	Ang2VEGF -0394	Ang2VEGF -0395





	No VH/VL exchange uncrossed	H/VL 1ge – ssed	VH/VL exchange crossed	L exchange - crossed
	LC ANG-2	HC ANG-2	HC VEGF	LC VEGF
Ang2VEGF- 0273	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF- 0396	SEQ ID NO: 5	SEQ ID NO: 6	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF- 0397	SEQ ID NO: 5	SEQ ID NO: 7	SEQ ID NO: 3	seq id no: 4
Ang2VEGF- 0394	SEQ ID NO: 8	SEQ ID NO: 6	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF- 0395	SEQ ID NO: 8	SEQ ID NO: 7	SEQ ID NO: 3	SEQ ID NO: 4



7 / 17

									¢ ¢
		NHV oN	No VH/VL exchange uncrossed	Je –		VHV	VH/VL_exchange crossed	- Jude -	
	CL isotype	CL ANG-2	CL ANG-2	CH1 ANG-2	CH1 ANG-2	CL isotype	CH1 VEGF	CL	Bence-Jones mispairing (VL-CH1/VL-CL-Pairing) (amount antibody with 2 Ang2-LC's) % by MS
Ang2VEGF- 0273	kappa	wt: Q124	wt; E123	wt: K147	wt: K213	kappa	wt	wt: Q124	~ <u>~~~</u>
Ang2VEGF- 0274	kappa	Q124K	E123K	K147E	K213E	kappa	wt	wt	o
Ang2VEGF- 0282	kappa	Q124R	E123K	K147E	K213E	kappa	wt	Q124E	D
Ang2VEGF- 0283	lambda (wt lambda: E124/ E123)	E124K	E123K	K147E	K213E	kappa	wt	wt:	Ð
Ang2VEGF- 0284	kappa	Q124R	E123K	K147E	K213D	kappa	wt	wt	O
Ang2VEGF- 0285	kappa	Q124R	E123K	K147E	K213D	kappa.	wt	Q124E	O
Ang2VEGF- 0286	kappa	Q124K	E123K	K147E	K213E	kappa	wt	Q124E	0

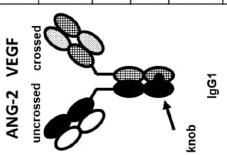
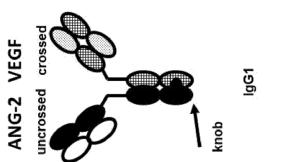


Fig. 5B

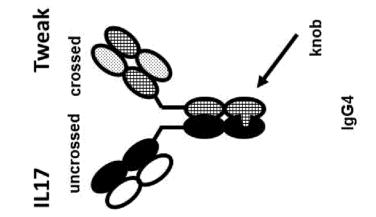
- FC		ge – sed Hrang,	vп/vL excnange - crossed	cnange -
		HC ANG-2	5	crossed
		-	HC VEGF	LC VEGF
Ang2VEGF-0273 SEQ ID NO: 1		SEQ ID NO: 2	SEQ ID NO: 3	seq id No: 4
Ang2VEGF-0274 SEQ.ID NO: 9		SEQ ID NO: 10 SEQ ID NO: 3	SEQ ID NOT3	SEQ ID NO: 4
Ang2VEGF-0282 SEQ ID NO: 11		SEQ ID NO: 10 SEQ ID NO: 3	seq id No: 3	SEQ ID NO: 12
Ang2VEGF-0283	SEQ ID NO: 13	SEQ.ID.NO::10 SEQ.ID.NO::3	SEQ.ID NO:3	SEQ ID NO: 4
Ang2VEGF-0284 SEQ ID NO: 11		SEQ ID NO: 14 SEQ ID NO: 3	SEQ ID NO:3	SEQ ID NO: 4
Ang2VEGF-0285 SEQ ID NO: 11		SEQ ID NO: 14 SEQ ID NO: 3	SEQ ID NO: 3	SEQ ID NO: 12
Ang2VEGF-0286 SEQ ID N0: 9		SEQ ID NO: 10 SEQ ID NO: 3	SEQ ID NO:3	SEQ ID NO: 12

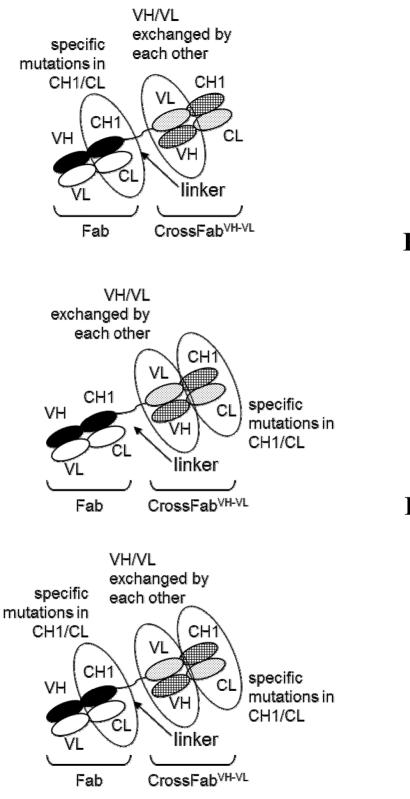


										Se de la constante de la const	F
				No VH/VL exchange uncrossed	H/VL exchange – uncrossed		/H/	VH/VL exchange crossed	ge -	**	
IL17 Tweak uncrossed crossed		CL isotype	CL IL17 (position 124)	CL IL17 (position 123)	CH1 IL17 (position 147)	CH1 IL17 (position 213)	CL isotype	CH1 TWEAK	CL TWEAK (position 124)	Bence-Jones mispairing (VL-CH1/VL-CL- Pairing) (amount antibody with 2 1L17-LC's) % by MS	
	TweaklL 17-0096	kappa	wt: Q124	wt: E123	wt: K147	wt: K213	kappa	wt	wt: Q124	~20%	
	TweaklL 17-0097	kappa	Q124K	E123R	K147E	K213E	kappa	wt	Q124E	0	
	TweaklL 17-0098	kappa	Q124K	E123R	K147E	K213D	kappa	wt	wt	O	
knob IgG4	TweaklL 17-0099	kappa	Q124K	E123R	K147E	K213D	kappa	wt	Q124E	0	
	TweaklL 17-0100	kappa	Q124K	E123K	K147E	K213E	kappa	wt	Q124E	0	
	TweaklL 17-0101	kappa	Q124K	E123K	K147E	K213E	kappa	ň	w	not determ.	

PCT/EP2015/057165

	No VH/VL exchange - uncrossed	exchange ssed	VH/VL exchange crossed	L exchange - crossed
	LC IL17	HCIL17	HCTweak	LC Tweak
TweakIL17-0096	SEQ ID NO: 15	SEQ ID NO: 16 SEQ ID NO: 17	SEQ ID NO: 17	SEQ ID NO: 18
TweakIL17-0097	SEQ ID NO: 19	SEQ ID NO: 20 SEQ ID NO: 17	SEQ ID NO: 17	SEQ ID NO: 21
TweakIL17-0098	SEQ ID NO: 19 SEQ ID NO: 22 SEQ ID NO: 17	SEQ ID NO: 22	SEQ ID NO: 17	SEQ ID NO: 18
TweaklL17-0099	SEQ ID NO: 19	SEQ ID NO: 22 SEQ ID NO: 17	SEQ ID NO: 17	SEQ ID NO: 21
TweakIL17-0100	SEQ ID NO: 23	SEQ ID NO: 20 SEQ ID NO: 17	SEQ ID NO: 17	SEQ ID NO: 21
TweaklL17-0101	SEQ ID NO: 23 SEQ ID NO: 20 SEQ ID NO: 17	SEQ ID NO: 20		SEQ ID NO: 18

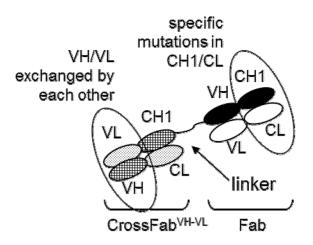




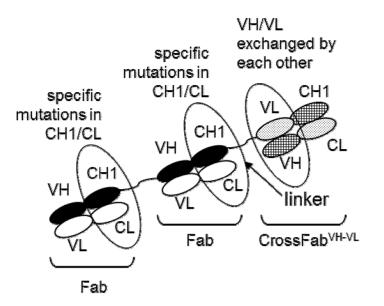




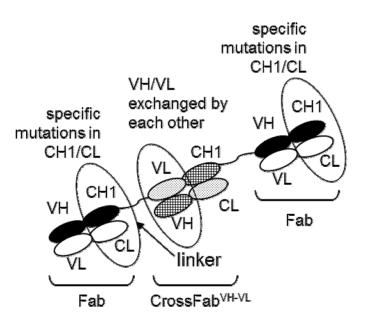














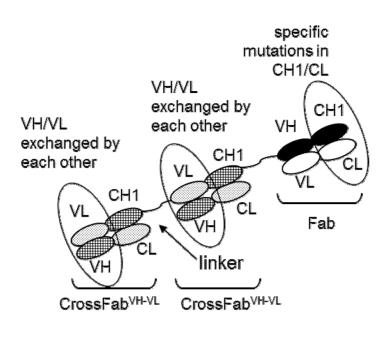
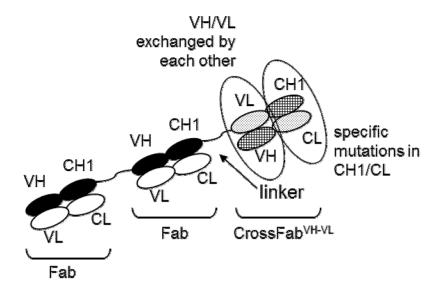


Fig. 8C





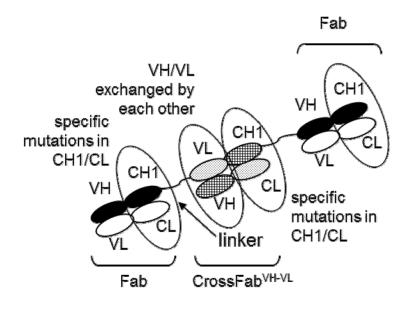


Fig. 8E

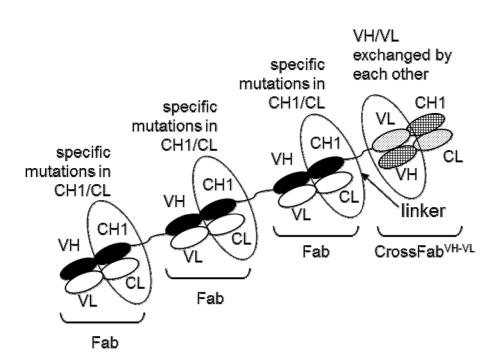


Fig. 9A

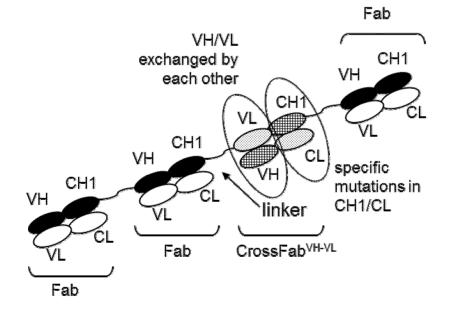


Fig. 9B

SEQUENCE LISTING <110> F. Hoffmann-La Roche AG <120> Multispecific antibodies <130> P32061-W0 <150> EP 14163165.5 2014-04-02 <151> <150> EP 14179034.5 <151> 2014-07-30 <160> 47 <170> PatentIn version 3.5 <210> 1 215 <211> <212> PRT Artificial <213> <220> light chain (LC) <Ang-2> wild type (wt) <223> <400> 1 GIn Pro Gly Leu Thr GIn Pro Pro Ser Val Ser Val Ala Pro Gly GIn 10 15 1 Thr Ala Arg IIe Thr Cys Gly Gly Asn Asn IIe Gly Ser Lys Ser Val His Trp Tyr GIn GIn Lys Pro GIy GIn Ala Pro Val Leu Val Val Tyr 35 40 45 Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser 50 55 60 Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly 65 70 75 80 65 Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His 90 95 Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Arg Thr Val Ala 100 105 110 Ala Pro Ser Val Phe IIe Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser 115 120 125 Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu 130 135 140 130 140 Ala Lys Val Gin Trp Lys Val Asp Asn Ala Leu Gin Ser Giy Asn Ser 145 150 155 160

eolf-seql.txt

eolf-seql.txt GIn Glu Ser Val Thr Glu GIn Asp Ser Lys Asp Ser Thr Tyr Ser Leu 165 170 175 Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val 185 180 Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys 195 200 205 Ser Phe Asn Arg Gly Glu Cys 210 <210> 2 <211> 457 <212> PRT Artificial <213> <220> <223> heavy chain (HC) <Ang-2> wild type (wt) <400> 2 GIn Val GIn Leu Val GIu Ser GIy Ala GIu Val Lys Lys Pro GIy Ala 1 5 10 15 10 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr 20 25 30 Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met 35 40 45 Gly Trp IIe Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe 50 60 GIn GIy Arg Val Thr Met Thr Arg Asp Thr Ser IIe Ser Thr Ala Tyr 65 70 75 80 Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys 90 95 Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr 100 105 110 Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser 115 120 125 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser 130 135 140 Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp 145 150 155 160 Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr 170 165 175 Page 2

Ser Gly Va	l His Th 180	- Phe	Pro	Al a	Val 185	Leu	GI n	Ser	Ser	GI y 190	Leu	Tyr
Ser Leu Se 19		Val	Thr	Val 200	Pro	Ser	Ser	Ser	Leu 205	GI y	Thr	Gl n
Thr Tyr II 210	e Cys Asi	n Val	Asn 215	Hi s	Lys	Pro	Ser	Asn 220	Thr	Lys	Val	Asp
Lys Lys Va 225	l Glu Pro	230 Lys	Ser	Cys	Asp	Lys	Thr 235	Hi s	Thr	Cys	Pro	Pro 240
Cys Pro Al	a Pro Glu 24		Leu	GI y	GI y	Pro 250	Ser	Val	Phe	Leu	Phe 255	Pro
Pro Lys Pr	o Lys Asj 260	o Thr	Leu	Met	IIе 265	Ser	Arg	Thr	Pro	GI u 270	Val	Thr
Cys Val Va 27		o Val	Ser	Hi s 280	GI u	Asp	Pro	GI u	Val 285	Lys	Phe	Asn
Trp Tyr Va 290	I Asp GI	y Val	GI u 295	Val	Hi s	Asn	AI a	Lys 300	Thr	Lys	Pro	Arg
Glu Glu Gl 305	n Tyr Asi	ו Ser 310	Thr	Tyr	Arg	Val	Val 315	Ser	Val	Leu	Thr	Val 320
Leu His Gl	n Asp Trj 32		Asn	GI y	Lys	GI u 330	Tyr	Lys	Cys	Lys	Val 335	Ser
Asn Lys Al	a Leu Pro 340	o Ala	Pro	lle	GI u 345	Lys	Thr	lle	Ser	Lys 350	Al a	Lys
GlyGlnPr 35		ı Pro	GI n	Val 360	Tyr	Thr	Leu	Pro	Pro 365	Cys	Arg	Asp
GLU Leu Th 370	r Lys Asi	ו GI n	Val 375	Ser	Leu	Trp	Cys	Leu 380	Val	Lys	GI y	Phe
Tyr Pro Se 385	r Asp II.	e Ala 390	Val	GI u	Trp	GI u	Ser 395	Asn	GI y	GI n	Pro	GI u 400
Asn Asn Ty	r Lys Th 40		Pro	Pro	Val	Leu 410	Asp	Ser	Asp	GI y	Ser 415	Phe
Phe Leu Ty	r Ser Ly: 420	s Leu	Thr	Val	Asp 425	Lys	Ser	Arg	Trp	GI n 430	GI n	GI y
Asn Val Ph 43	e Ser Cys 5	s Ser	Val	Met 440	Hi s		Ala ge 3	Leu	Hi s 445	Asn	Hi s	Tyr

eolf-seql.txt

Thr GIn Lys Ser Leu Ser Leu Ser Pro 450 455 <210> 3 <211> <212> 437 PRT <213> Artificial <220> <223> heavy chain (HC) <VEGF> with VH-VL exchange wild type (wt) <400> 3 Asp Ile GIn Met Thr GIn Ser Pro Ser Ser Leu Ser Ala Ser Val GIy 1 5 10 15 Asp Arg Val Thr IIe Thr Cys Ser Ala Ser GIn Asp IIe Ser Asn Tyr 20 25 30 20 Leu Asn Trp Tyr GIn GIn Lys Pro GIy Lys Ala Pro Lys Val Leu IIe 35 40 45 Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly 50 55 60 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr IIe Ser Ser Leu Gln Pro 65 70 75 80 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp 85 95 90 Thr Phe Gly Gln Gly Thr Lys Val Glu IIe Lys Ser Ser Ala Ser Thr 100 105 110 Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser 115 120 125 Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu 130 135 140 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His 145 150 155 160 Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser 165 170 175 165 175 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr IIe Cys 180 185 190 180 Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu 195 200 205

Page 4

eolf-seql.txt Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Al a Pro 210 215 220 215 Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys 230 225 235 Asp Thr Leu Met IIe Ser Arg Thr Pro Glu Val Thr Cys Val Val Val 245 250 255 Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp 260 265 270 265 260 Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr 275 280 285 Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp 290 295 300 Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu 305 310 315 320 Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg 325 330 335 335 Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys 340 345 350 Asn GIn Val Ser Leu Ser Cys Ala Val Lys GIy Phe Tyr Pro Ser Asp 355 360 365 lle Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys 370 375 380 Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val 385 390 395 Ser 385 390 400 Lys Leu Thr Val Asp Lys Ser Arg Trp Gin Gin Giy Asn Val Phe Ser 405 410 415 Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser 420 425 430 Leu Ser Leu Ser Pro 435 <210> 4 <211> 230 PRT <212> <213> Artificial <220> light chain (LC) <VEGF> with VH-VL exchange wild type (wt) <223>

eolf-seql.txt <400> Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly 1 5 10 15 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr 20 25 30 Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35 40 45 Gly Trp IIe Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe 50 55 60 Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr 65 70 75 80 Leu GIn Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys 85 90 95 Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val 100 105 110 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Val Ala Ala 115 120 125 Pro Ser Val Phe IIe Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly 130 135 140 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala145150150155160 145 155 160 Lys Val GIn Trp Lys Val Asp Asn Ala Leu GIn Ser GIy Asn Ser GIn 165 170 175 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser 180 185 190 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr 195 200 205 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser 210 215 220 Phe Asn Arg Gly Glu Cys 225 5 215 <210> <211> PRT <212> <213> Artificial <220>

eolf-seql.txt light chain (LC) <Ang-2> with Q124K substitution <223> <400> 5 GIN Pro GIY Leu Thr GIN Pro Pro Ser Val Ser Val Ala Pro GIY GIN 1 5 10 15 Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val His Trp Tyr GIn GIn Lys Pro GIy GIn Ala Pro Val Leu Val Val Tyr 35 40 45 Asp Asp Ser Asp Arg Pro Ser Gly IIe Pro Glu Arg Phe Ser Gly Ser 50 60 Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly 65 70 75 80 65 Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His 85 90 95 Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Arg Thr Val Ala 100 105 110 Ala Pro Ser Val Phe IIe Phe Pro Pro Ser Asp Glu Lys Leu Lys Ser 115 120 125 Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu 130 135 140 Ala Lys Val Gin Trp Lys Val Asp Asn Ala Leu Gin Ser Giy Asn Ser 145 150 155 160 145 GIn Glu Ser Val Thr Glu GIn Asp Ser Lys Asp Ser Thr Tyr Ser Leu 165 170 175 Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val 180 185 190 Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys 195 200 205 Ser Phe Asn Arg Gly Glu Cys 210 215 <210> 6 457 <211> <212> PRT <213> Artificial <220> heavy chain (HC) <Ang-2> with K147E substitution <223>

eolf-seql.txt <400> 6 GIN VAL GIN Leu VAL GIN Ser GLY ALA GIN VAL Lys Lys Pro GLY ALA 1 5 10 15 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr 20 25 30 Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met 35 40 45 Gly Trp IIe Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe 50 55 60 GIn GIy Arg Val Thr Met Thr Arg Asp Thr Ser IIe Ser Thr Ala Tyr 65 70 75 80 Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys 85 90 95 Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr 100 105 110 100 Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser 115 120 125 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser 130 135 140 Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Glu Asp 145 150 155 160 Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr 165 170 175 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr 180 185 190 Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln 195 200 205 Thr Tyr IIe Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp 210 215 220 Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro 225 230 235 240 240 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro 245 250 255 Pro Lys Pro Lys Asp Thr Leu Met IIe Ser Arg Thr Pro Glu Val Thr 260 265 270 Page 8

Cys Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn 275 280 285
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg 290 295 300
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val 305 310 315 320
Leu His GIn Asp Trp Leu Asn GIy Lys GIu Tyr Lys Cys Lys Val Ser 325 330 335
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys 340 345 350
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp 355 360 365
Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe 370 375 380
Tyr Pro Ser Asp IIe Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu 385 390 395 400
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe 405 410 415
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp GIn GIn GIy 420 425 430
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr 435 440 445
Thr Gln Lys Ser Leu Ser Leu Ser Pro 450 455
<210> 7 <211> 457 <212> PRT <213> Artificial
<220>
<223> heavy chain (HC) <ang-2> with K213E substitution <400> 7</ang-2>
GIn Val GIn Leu Val GIu Ser GIy Ala GIu Val Lys Lys Pro GIy Ala 1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr 20 25 30

eolf-seql.txt Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met 35 40 40 Gly Trp IIe Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe 50 60 GIn Gly Arg Val Thr Met Thr Arg Asp Thr Ser IIe Ser Thr Ala Tyr 65 70 75 80 Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys 85 90 95 Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr 100 105 110 100 Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser 115 120 125 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser 130 135 140 Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp 145 150 155 160 Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr 165 170 175 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr 180 185 190 180 Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln 195 200 205Thr Tyr IIe Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp 210 215 220 Glu Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro 225 230 235 240 240 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro 245 250 250 255 Pro Lys Pro Lys Asp Thr Leu Met IIe Ser Arg Thr Pro Glu Val Thr 260 265 270 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn 275 280 285 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg 290 295 300

eolf-seql.txt Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val 305 310 315 320 Leu His GIn Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser 325 330 335 Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys 340 345 350 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp 355 360 365 Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe 370 375 380 Tyr Pro Ser Asp IIe Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu 385 390 395 400 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe 405 410 415 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly 420 425 430 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr 440 435 445 Thr GIn Lys Ser Leu Ser Leu Ser Pro 450 455 <210> 8 215 <211> <212> PRT <213> Artificial <220> light chain (LC) <Ang-2> with E123K substitution <223> <400> 8 GIn Pro Gly Leu Thr GIn Pro Pro Ser Val Ser Val Ala Pro Gly GIn 10 Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val 20 25 30 His Trp Tyr GIn GIn Lys Pro GIy GIn Ala Pro Val Leu Val Val Tyr 35 40 45 45 Asp Asp Ser Asp Arg Pro Ser Gly IIe Pro Glu Arg Phe Ser Gly Ser 50 60 Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly 65 70 75 80 65 Page 11

eolf-seql.txt

Asp Glu Ala	Asp Ty 85		Cys	GI n	Val	Trp 90	Asp	Ser	Ser	Ser	Asp 95	Hi s
Tyr Val Phe	GI y Th 100	ır Gly	Thr	Lys	Val 105	Thr	Val	Leu	Arg	Thr 110	Val	Al a
Ala Pro Ser 115	Val Pł	e lle	Phe	Pro 120	Pro	Ser	Asp	Lys	GI n 125	Leu	Lys	Ser
Gly Thr Ala 130	Ser Va	l Val	Cys 135	Leu	Leu	Asn	Asn	Phe 140	Tyr	Pro	Arg	GI u
Ala Lys Val 145	GIn Tr	p Lys 150		Asp	Asn	Al a	Leu 155	GI n	Ser	GI y	Asn	Ser 160
Gln Glu Ser	Val Th 16		Gl n	Asp	Ser	Lys 170	Asp	Ser	Thr	Tyr	Ser 175	Leu
Ser Ser Thr	Leu Th 180	ir Leu	Ser	Lys	AI a 185	Asp	Tyr	GI u	Lys	Hi s 190	Lys	Val
Tyr Ala Cys 195	Glu Va	l Thr	Hi s	GI n 200	GI y	Leu	Ser	Ser	Pro 205	Val	Thr	Lys
Ser Phe Asn 210	Arg GI	y Glu	Cys 215									
<210> 9 <211> 215 <212> PRT <213> Arti	fi ci al											
	t chair titutic		<an< td=""><td>g-2></td><td>wi tł</td><td>012 n</td><td>24K s</td><td>subst</td><td>ti tu†</td><td>tion</td><td>and</td><td>E123K</td></an<>	g-2>	wi tł	012 n	24K s	subst	ti tu†	tion	and	E123K
<400> 9												
GIn Pro GIy 1	Leu Tr 5	ır Gln	Pro	Pro	Ser	Val 10	Ser	Val	AI a	Pro	GI y 15	Gl n
Thr Ala Arg	lle Th 20	ır Cys	GI y	GI y	Asn 25	Asn	lle	GI y	Ser	Lys 30	Ser	Val
His Trp Tyr			Dro	GLV	GLn	Δla	Pro	Val	Leu	Val	Val	Tyr
35	GIn GI	n Lys	FIU	40	0111	Al d			45			5
		-		40					45			-

Page 12

							eo	lf-s	eqI.	txt				
Asp GI	u Ala	Asp	Tyr 85	Tyr	Cys	GI n	Val	Trp 90	Asp	Ser	Ser	Ser	Asp 95	Hi s
Tyr Va	Phe	GI y 100	Thr	GI y	Thr	Lys	Val 105	Thr	Val	Leu	Arg	Thr 110	Val	Ala
Ala Pr	o Ser 115		Phe	lle	Phe	Pro 120	Pro	Ser	Asp	Lys	Lys 125	Leu	Lys	Ser
GI y Th 13		Ser	Val	Val	Cys 135	Leu	Leu	Asn	Asn	Phe 140	Tyr	Pro	Arg	GI u
ALa Ly 145	s Val	GI n	Trp	Lys 150	Val	Asp	Asn	Al a	Leu 155	GI n	Ser	GI y	Asn	Ser 160
GIn GI	u Ser	Val	Thr 165	GI u	GI n	Asp	Ser	Lys 170	Asp	Ser	Thr	Tyr	Ser 175	Leu
Ser Se	r Thr	Leu 180	Thr	Leu	Ser	Lys	AI a 185	Asp	Tyr	GI u	Lys	Hi s 190	Lys	Val
Tyr Al	a Cys 195		Val	Thr	Hi s	GI n 200	GI y	Leu	Ser	Ser	Pro 205	Val	Thr	Lys
Ser Ph 21		Arg	GI y	GI u	Cys 215									
<210> <211> <212> <213>	10 457 PRT Arti	ficia	al											
<220> <223>	heav subs			(HC)	<ang< td=""><td>g-2></td><td>wi tł</td><td>ר K1⊿</td><td>47E s</td><td>subst</td><td>titu</td><td>tion</td><td>and</td><td>K213E</td></ang<>	g-2>	wi tł	ר K1⊿	47E s	subst	titu	tion	and	K213E
<400>	10													
GIn Va 1	GIn	Leu	Val 5	GI u	Ser	GI y	Al a	GI u 10	Val	Lys	Lys	Pro	GI y 15	Ala
Ser Va	l Lys	Val 20	Ser	Cys	Lys	Al a	Ser 25	GI y	Tyr	Thr	Phe	Thr 30	GI y	Tyr
Tyr Me	tHis 35	Тгр	Val	Arg	GI n	AI a 40	Pro	GI y	GI n	GI y	Leu 45	GI u	Trp	Met
GIy Tr 50	o Ile	Asn	Pro	Asn	Ser 55	GI y	GI y	Thr	Asn	Tyr 60	AI a	GI n	Lys	Phe
GIn GI 65	y Arg	Val	Thr	Met 70	Thr	Arg	Asp	Thr	Ser 75	lle	Ser	Thr	Al a	Tyr 80

eolf-seql.txt Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys 85 90 95 Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr 100 105 110 100 Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser 115 120 125 125 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser 130 135 140 Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Glu Asp 145 150 155 160 Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr 165 170 175 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr 185 190 180 Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln 195 200 205 Thr Tyr IIe Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp 210 215 220 Glu Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro 225 230 235 240 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro 245 250 255 245 Pro Lys Pro Lys Asp Thr Leu Met IIe Ser Arg Thr Pro Glu Val Thr 260 265 270 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn 275 280 285 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg 290 295 300 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val 305 310 315 320 Leu His GIn Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser 325 330 335 Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys 340 345 350

eolf-seql.txt Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp 355 360 365 Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe 375 370 380 Tyr Pro Ser Asp IIe 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 405 410 415 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly 420 425 430 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr 435 440 445 Thr GIn Lys Ser Leu Ser Leu Ser Pro 450 455 <210> 11 215 <211> <212> PRT Artificial <213> <220> light chain (LC) <Ang-2> with Q124R substitution and E123K <223> substitution <400> 11 GIN Pro GIY Leu Thr GIN Pro Pro Ser Val Ser Val Ala Pro GIY GIN 1 5 10 15 Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val 20 25 30 His Trp Tyr GIn GIn Lys Pro Gly GIn Ala Pro Val Leu Val Val Tyr 35 40 45 45 Asp Asp Ser Asp Arg Pro Ser Gly IIe Pro Glu Arg Phe Ser Gly Ser 50 60 Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly 65 70 75 80 70 65 Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His 85 90 95 Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Arg Thr Val Ala 100 105 110 Ala Pro Ser Val Phe IIe Phe Pro Pro Ser Asp Lys Arg Leu Lys Ser Page 15

eolf-seql.txt 120 115 125 Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu 130 135 140 Ala Lys Val Gin Trp Lys Val Asp Asn Ala Leu Gin Ser Giy Asn Ser 145 150 155 160 160 GIn Glu Ser Val Thr Glu GIn Asp Ser Lys Asp Ser Thr Tyr Ser Leu 165 170 175 Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val 180 185 190 Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys 195 200 205 Ser Phe Asn Arg Gly Glu Cys 210 <210> 12 <211> 230 <212> PRT Artificial <213> <220> light chain (LC) <VEGF> with Q124E substitution <223> <400> 12 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly 10 15 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr 20 25 30 Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35 40 45 Gly Trp IIe Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe 50 55 60 Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr 65 70 75 80 Leu GIn Met Asn Ser Leu Arg Ala GIu Asp Thr Ala Val Tyr Tyr Cys 85 90 95 Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val 100 105 110 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Val Ala Ala 115 120 125

					eo	lf-s	eqI.	txt				
Pro Ser Val 130	Phe II e	e Phe	Pro 135	Pro	Ser	Asp	GI u	GI u 140	Leu	Lys	Ser	GI y
Thr Ala Ser 145	Val Val	Cys 150	Leu	Leu	Asn	Asn	Phe 155	Tyr	Pro	Arg	GI u	AI a 160
Lys Val Gln	Trp Lys 165		Asp	Asn	Al a	Leu 170	GI n	Ser	GI y	Asn	Ser 175	Gl n
Glu Ser Val	Thr Glu 180	ıGIn	Asp	Ser	Lys 185	Asp	Ser	Thr	Tyr	Ser 190	Leu	Ser
Ser Thr Leu 195	Thr Leu	ı Ser	Lys	AI a 200	Asp	Tyr	GI u	Lys	Hi s 205	Lys	Val	Tyr
Ala Cys Glu 210	Val Thr	His	GI n 215	GI y	Leu	Ser	Ser	Pro 220	Val	Thr	Lys	Ser
Phe Asn Arg 225	Gly Glu	ı Cys 230										
<210> 13 <211> 214 <212> PRT												
<213> Arti	ficial											
<220> <223> ligh [.]	ficial t chain titutior		<anç< td=""><td>g-2></td><td>wi tł</td><td>n E12</td><td>24K s</td><td>subst</td><td>ti tu</td><td>tion</td><td>and</td><td>E123K</td></anç<>	g-2>	wi tł	n E12	24K s	subst	ti tu	tion	and	E123K
<220> <223> ligh [.]	t chain		<anç< td=""><td>g-2></td><td>witł</td><td>n E12</td><td>24K s</td><td>subst</td><td>ti tu†</td><td>ti on</td><td>and</td><td>E123K</td></anç<>	g-2>	witł	n E12	24K s	subst	ti tu†	ti on	and	E123K
<220> <223> Iigh subs	t chain titutior	ו		-								
<220> <223> ligh subs <400> 13 Gln Pro Gly	t chain titutior Leu Thi 5	GIN	Pro	Pro	Ser	Val 10	Ser	Val	AI a	Pro	GI y 15	GI n
<220> <223> ligh subs <400> 13 GIn Pro GIy 1	t chain titutior Leu Thr 5 Ile Thr 20	GIN Cys	Pro Gl y	Pro Gl y	Ser Asn 25	Val 10 Asn	Ser IIe	Val Gl y	Al a Ser	Pro Lys 30	GI y 15 Ser	GI n Val
<220> <223> ligh: subs <400> 13 GIn Pro GIy 1 Thr Ala Arg His Trp Tyr	t chain titutior Leu Thr 5 Ile Thr 20 Gln Glr	GIN Cys Lys	Pro Gl y Pro	Pro Gl y Gl y 40	Ser Asn 25 GI n	Val 10 Asn Al a	Ser IIe Pro	Val Gl y Val	Al a Ser Leu 45	Pro Lys 30 Val	GI y 15 Ser Val	GI n Val Tyr
<220> <223> ligh: subs <400> 13 GIn Pro GIy 1 Thr Ala Arg His Trp Tyr 35 Asp Asp Ser	t chain titution Leu Thu 5 Ile Thu 20 Gln Glu Asp Arg	GIN Cys Lys Pro	Pro Gl y Pro Ser 55	Pro Gly Gly 40 Gly	Ser Asn 25 GI n II e	Val 10 Asn Al a Pro	Ser IIe Pro GIu	Val Gly Val Arg 60	Al a Ser Leu 45 Phe	Pro Lys 30 Val Ser	GI y 15 Ser Val GI y	GI n Val Tyr Ser
<220> <223> light subst <400> 13 GIn Pro GIy 1 Thr Ala Arg His Trp Tyr 35 Asp Asp Ser 50 Asn Ser GIy	t chain titutior Leu Thr 5 Ile Thr 20 GIn GIr Asp Arg Asn Thr	GIN Cys Lys Pro Ala 70	Pro Gl y Pro Ser 55 Thr	Pro Gly Gly 40 Gly Leu	Ser Asn 25 GI n II e Thr	Val 10 Asn Al a Pro I I e	Ser IIe Pro Glu Ser 75	Val Gl y Val Arg 60 Arg	Al a Ser Leu 45 Phe Val	Pro Lys 30 Val Ser Glu	GI y 15 Ser Val GI y AI a	GI n Val Tyr Ser GI y 80

eolf-seql.txt Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Lys Lys Leu Gln 120 125 Ala Asn Lys Ala Thr Leu Val Cys Leu IIe Ser Asp Phe Tyr Pro Gly 130 135 140 Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala Gly 145 150 155 160 16Ŏ Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala 165 170 175 165 Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg Ser 185 190 Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val 195 200 205 Ala Pro Thr Glu Cys Ser 210 <210> 14 <211> 457 <212> PRT Artificial <213> <220> heavy chain (HC) <Ang-2> with K147E substitution and K213D <223> substitution <400> 14 GIN VAL GIN Leu VAL GIU Ser GLY ALA GLU VAL Lys Lys Pro GLY ALA 1 5 10 15 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr 20 25 30 Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met 35 40 45 Gly Trp IIe Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe 50 60 GIn GIy Arg Val Thr Met Thr Arg Asp Thr Ser IIe Ser Thr Ala Tyr 65 70 75 80 Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys 85 90 95 Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr 100 105 110 Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Page 18

	115					120	eo	lf-s	eqI .	txt	125			
Ser Ala 130	Ser	Thr	Lys	GI y	Pro 135	Ser	Val	Phe	Pro	Leu 140	Al a	Pro	Ser	Ser
Lys Ser 145	Thr	Ser	GI y	GI y 150	Thr	Al a	Al a	Leu	GI y 155	Cys	Leu	Val	GI u	Asp 160
Tyr Phe	Pro	GI u	Pro 165	Val	Thr	Val	Ser	Trp 170	Asn	Ser	GI y	Al a	Leu 175	Thr
Ser Gly	Val	Hi s 180	Thr	Phe	Pro	Al a	Val 185	Leu	GI n	Ser	Ser	GI y 190	Leu	Tyr
Ser Leu	Ser 195	Ser	Val	Val	Thr	Val 200	Pro	Ser	Ser	Ser	Leu 205	GI y	Thr	Gl n
Thr Tyr 210	lle	Cys	Asn	Val	Asn 215	Hi s	Lys	Pro	Ser	Asn 220	Thr	Lys	Val	Asp
Asp Lys 225	Val	GI u	Pro	Lys 230	Ser	Cys	Asp	Lys	Thr 235	Hi s	Thr	Cys	Pro	Pro 240
Cys Pro	Al a	Pro	Gl u 245	Leu	Leu	GI y	GI y	Pro 250	Ser	Val	Phe	Leu	Phe 255	Pro
Pro Lys	Pro	Lys 260	Asp	Thr	Leu	Met	Пе 265	Ser	Arg	Thr	Pro	GI u 270	Val	Thr
	Val 275	Val	Asp	Val	Ser	Hi s 280	GI u	Asp	Pro	GI u	Val 285	Lys	Phe	Asn
Trp Tyr 290	Val	Asp	GI y	Val	GI u 295	Val	Hi s	Asn	Al a	Lys 300	Thr	Lys	Pro	Arg
GluGlu 305	GI n	Tyr	Asn	Ser 310	Thr	Tyr	Arg	Val	Val 315	Ser	Val	Leu	Thr	Val 320
Leu His	GI n	Asp	Trp 325	Leu	Asn	GI y	Lys	GI u 330	Tyr	Lys	Cys	Lys	Val 335	Ser
Asn Lys	Al a	Leu 340	Pro	Al a	Pro	lle	GI u 345	Lys	Thr	lle	Ser	Lys 350	Al a	Lys
Gly Gln	Pro 355	Arg	GI u	Pro	Gl n	Val 360	Tyr	Thr	Leu	Pro	Pro 365	Cys	Arg	Asp
GLU Leu 370	Thr	Lys	Asn	Gl n	Val 375	Ser	Leu	Trp	Cys	Leu 380	Val	Lys	GI y	Phe
Tyr Pro	Ser	Asp	lle	Al a	Val	GI u	Trp		Ser e 19		GI y	Gl n	Pro	Glu

eolf-seql.txt 390 385 395 400 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe 405 410 415 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly 420 425 430 430 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr 435 440 445 Thr GIn Lys Ser Leu Ser Leu Ser Pro 455 450 <210> 15 <211> 219 <212> PRT Artificial <213> <220> light chain (LC) <IL-17> wild type (wt) <223> <400> 15 Aspile Val Met Thr Gin Ser Pro Leu Ser Leu Pro Val Thr Pro Giy 5 10 15 Glu Pro Ala Ser IIe Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser 20 25 30 20 30 Asn Gly Asp Thr Tyr Phe His Trp Tyr Leu Gln Lys Pro Gly Gln Ser 35 40 45 Pro GIn Leu Leu IIe Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro 50 55 60 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys IIe 5 70 75 80 65 80 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Thr 85 90 95 Thr His Ala Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu IIe Lys 100 105 110 Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu 115 120 125 GIn Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe 130 135 140 Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln 145 150 155 160

eolf-seql.txt Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser 170 165 Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu 180 185 190 Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser 195 200 205 Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys 210 215 <210> 16 446 <211> <212> PRT Artificial <213> <220> <223> heavy chain (HC) <IL-17> wild type (wt) <400> 16 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly 1 5 10 15 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Asp Ser Tyr 20 25 30 Gly Val His Trp Val Arg Gln Ala Thr Gly Lys Gly Leu Glu Trp Val 35 40 45 Ser Val IIe Trp Ser Asp Gly Thr Thr Thr Tyr Asn Ser Ala Leu Lys 50 55 60 Ser Arg Phe Thr IIe Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr Leu 65 70 75 80 GIn Met Asn Ser Leu Arg Ala Gly Asp Thr Ala Val Tyr Tyr Cys Ala 85 90 95 Arg Asp Thr His Tyr Arg Leu Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly 100 105 110 GIn GIy Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys GIy Pro Ser 115 120 125 Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala 130 135 140 Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val 145 150 155 160 Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Page 21

	165	eolf-seql.txt 170	175
Val Leu Gln Ser		Tyr Ser Leu Ser Ser Val	Val Thr Val
180		185	190
Pro Ser Ser Ser 195	Leu Gly Thr	Lys Thr Tyr Thr Cys Asn 200 205	Val Asp His
Lys Pro Ser Asr	n Thr Lys Val	Asp Lys Arg Val Glu Ser	Lys Tyr Gly
210	215	220	
Pro Pro Cys Pro	Pro Cys Pro	Ala Pro Glu Phe Glu Gly	Gly Pro Ser
225	230	235	240
Val Phe Leu Phe	e Pro Pro Lys	Pro Lys Asp Thr Leu Met	lle Ser Arg
	245	250	255
Thr Pro Glu Val		Val Val Asp Val Ser Gln	Glu Asp Pro
260		265	270
Glu Val Gln Phé 275	e Asn Trp Tyr	Val Asp Gly Val Glu Val 280 285	His Asn Ala
Lys Thr Lys Pro	o Arg Glu Glu	GIn Phe Asn Ser Thr Tyr .	Arg Val Val
290	295	300	
Ser Val Leu Thr	Val Leu His	GIn Asp Trp Leu Asn GIy	Lys Glu Tyr
305	310	315	320
Lys Cys Lys Val	Ser Asn Lys	Gly Leu Pro Ser Ser Ile	Glu Lys Thr
	325	330	335
lle Ser Lys Ala		Pro Arg Glu Pro Gln Val	Cys Thr Leu
340		345	350
Pro Pro Ser Glr 355	n Glu Glu Met	Thr Lys Asn GIn Val Ser 360 365	Leu Ser Cys
Ala Val Lys Gly	Phe Tyr Pro	Ser Asp IIe Ala Val Glu	Trp Glu Ser
370	375	380	
Asn Gly Gln Pro	o Glu Asn Asn	Tyr Lys Thr Thr Pro Pro	Val Leu Asp
385	390	395	400
Ser Asp Gly Ser	Phe Phe Leu 405	Val Ser Arg Leu Thr Val 410	Asp Lys Ser 415
Arg Trp Gln Glu		Phe Ser Cys Ser Val Met	His Glu Ala
420		425	430
Leu His Asn His	s Tyr Thr Gln	Lys Ser Leu Ser Leu Ser Page 22	Leu

eol f-seql.txt 440 445

435

<210> 17 <211> 438 <212> PRT <213> Artificial <220> heavy chain (HC) <TWEAK> with VH-VL exchange wild type (wt) <223> <400> 17 Asp IIe GIn Met Thr GIn Ser Pro Ser Ser Val Ser Ala Ser Val Gly 1 5 10 15 Asp Arg Val Thr IIe Thr Cys GIn Ala Ser GIn Asn IIe Tyr Ser Asn 20 25 30 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu IIe 35 40 45 Tyr Thr Ala Ser Tyr Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly 50 55 60 Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr IIe Ser Ser Leu Gln Pro 65 70 75 80 65 80 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Thr Ala Tyr Tyr Asn Ser Arg 85 90 Pro Asp Thr Val Ala Phe Gly Gly Gly Thr Lys Val Glu IIe Lys Ser 100 105 110 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser 115 120 125 Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp 130 135 140 Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr 145 150 155 160 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr 165 170 175 Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys 180 185 190 Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp 195 200 205 Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Cys Pro Ala 210 215 220

eolf-seql.txt
Pro Glu Phe Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro 225 230 235 240
Lys Asp Thr Leu Met IIe Ser Arg Thr Pro Glu Val Thr Cys Val Val 245 250 255
Val Asp Val Ser Gin Giu Asp Pro Giu Val Gin Phe Asn Trp Tyr Val 260 265 270
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln 275 280 285
Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln 290 295 300
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly 305 310 315 320
Leu Pro Ser Ser IIe Glu Lys Thr IIe Ser Lys Ala Lys Gly Gln Pro 325 330 335
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Gln Glu Glu Met Thr 340 345 350
Lys Asn GIn Val Ser Leu Trp Cys Leu Val Lys GIy Phe Tyr Pro Ser 355 360 365
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr 370 375 380
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr 385 390 395 400
Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe 405 410 415
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys 420 425 430
Ser Leu Ser Leu 435
<210> 18 <211> 227 <212> PRT <213> Artificial
<220> <223> light chain (LC) <tweak> with VH-VL exchange wild type (wt)</tweak>
<400> 18
GIn Val GIn Leu Val GIn Ser GIy Ala GIu Val Lys Lys Pro GIy Ser Page 24

1	5	eolf-seql.txt 10	15
Ser Val Lys Val	Ser Cys Lys	Ala Ser Gly Phe Asp Phe	e Ser Thr Tyr
20		25	30
Tyr Met Ser Trp 35	Val Arg Gln	Ala Pro Gly Gln Gly Leu 40 45	ıGlu Trp Met
Gly Thr Val Tyr	Val Arg Gln	Gly Thr Thr Tyr Tyr Ala	a Ser Trp Leu
50	55	60	
Asn GLy Arg Val	Thr Ile Thr	Ala Asp Glu Ser Thr Ser	r Thr Ala Tyr
65	70	75	80
Met Glu Leu Ser	Ser Leu Arg	Ser Glu Asp Thr Ala Val	Tyr Tyr Cys
	85	90	95
Ala Arg Gly Gly		Asp Asp Ala Phe Val IIe	e Trp Gly Gln
100		105	110
Gly Thr Leu Val 115	Thr Val Ser	Ser Ala Ser Val Ala Ala 120 125	
Phe IIe Phe Pro	Pro Ser Asp	Glu Gln Leu Lys Ser Gly	/ Thr Ala Ser
130	135	140	
Val Val Cys Leu	Leu Asn Asn	Phe Tyr Pro Arg Glu Ala	a Lys Val Gln
145	150	155	160
Trp Lys Val Asp	Asn Ala Leu	Gln Ser Gly Asn Ser Glr	n Glu Ser Val
	165	170	175
Thr Glu Gln Asp		Ser Thr Tyr Ser Leu Ser	r Ser Thr Leu
180		185	190
Thr Leu Ser Lys 195	Ala Asp Tyr	Glu Lys His Lys Val Tyr 200 205	
Val Thr His Gln	Gly Leu Ser	Ser Pro Val Thr Lys Ser	- Phe Asn Arg
210	215	220	
Gly Glu Cys 225			
<210> 19 <211> 219 <212> PRT <213> Artificia	al		
<220> <223> light cha substitu		-17> with Q124K substitu	ution and E123R

eolf-seql.txt <400> 19 Asp IIe Val Met Thr GIn Ser Pro Leu Ser Leu Pro Val Thr Pro Gly 5 10 15 Glu Pro Ala Ser IIe Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser 20 25 30 30 Asn Gly Asp Thr Tyr Phe His Trp Tyr Leu Gln Lys Pro Gly Gln Ser 35 40 45 Pro GIn Leu Leu IIe Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro 50 55 60 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys IIe 65 70 75 80 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Thr 85 90 95 85 Thr His Ala Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu IIe Lys 100 105 110 Arg Thr Val Ala Ala Pro Ser Val Phe IIe Phe Pro Pro Ser Asp Arg 120 115 125 Lys Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe 130 135 140 Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln 145 150 155 160 Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser 170 165 Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu 180 185 190 Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser 195 200 205 Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys 210 215<210> 20 <211> 446 <212> PRT <213> Artificial <220> heavy chain (HC) <IL-17> with K147E substitution and K213E <223> substitution <400> 20

								eo	lf-s	eqI.	txt				
GI u 1	Val	GI n	Leu	Val 5	GI u	Ser	GI y	GI y	GI y 10	Leu	Val	GI n	Pro	GI y 15	GI y
Ser	Leu	Arg	Leu 20	Ser	Cys	Al a	Al a	Ser 25	GI y	Phe	Ser	Leu	Asp 30	Ser	Tyr
GI y	Val	His 35	Trp	Val	Arg	GI n	Al a 40	Thr	GI y	Lys	GI y	Leu 45	GI u	Trp	Val
Ser	Val 50	lle	Trp	Ser	Asp	GI y 55	Thr	Thr	Thr	Tyr	Asn 60	Ser	Al a	Leu	Lys
Ser 65	Arg	Phe	Thr	lle	Ser 70	Arg	GI u	Asn	AI a	Lys 75	Asn	Ser	Leu	Tyr	Leu 80
GI n	Met	Asn	Ser	Leu 85	Arg	Al a	GI y	Asp	Thr 90	Al a	Val	Tyr	Tyr	Cys 95	Al a
Arg	Asp	Thr	Hi s 100	Tyr	Arg	Leu	Tyr	Tyr 105	Tyr	Al a	Met	Asp	Tyr 110	Trp	GI y
Gl n	GI y	Thr 115	Thr	Val	Thr	Val	Ser 120	Ser	Al a	Ser	Thr	Lys 125	GI y	Pro	Ser
Val	Phe 130	Pro	Leu	AI a	Pro	Cys 135	Ser	Arg	Ser	Thr	Ser 140	GI u	Ser	Thr	Al a
AI a 145	Leu	GI y	Cys	Leu	Val 150	GI u	Asp	Tyr	Phe	Pro 155	GI u	Pro	Val	Thr	Val 160
Ser	Trp	Asn	Ser	GI y 165	Al a	Leu	Thr	Ser	GI y 170	Val	Hi s	Thr	Phe	Pro 175	Al a
Val	Leu	GI n	Ser 180	Ser	GI y	Leu	Tyr	Ser 185	Leu	Ser	Ser	Val	Val 190	Thr	Val
Pro	Ser	Ser 195	Ser	Leu	GI y	Thr	Lys 200	Thr	Tyr	Thr	Cys	Asn 205	Val	Asp	Hi s
Lys	Pro 210	Ser	Asn	Thr	Lys	Val 215	Asp	GI u	Arg	Val	GI u 220	Ser	Lys	Tyr	GI y
Pro 225	Pro	Cys	Pro	Pro	Cys 230	Pro	Al a	Pro	GI u	Phe 235	GI u	GI y	GI y	Pro	Ser 240
Val	Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp 250	Thr	Leu	Met	lle	Ser 255	Arg
Thr	Pro	GI u	Val 260	Thr	Cys	Val	Val	Val 265	Asp	Val	Ser	GI n	GI u 270	Asp	Pro

Page 27

	eol f-seq	I.txt
Glu Val Gln Phe Asn ⁻	Trp Tyr Val Asp Gly Va	al Glu Val His Asn Ala
275	280	285
Lys Thr Lys Pro Arg (Glu Glu Gln Phe Asn Se	er Thr Tyr Arg Val Val
290	295	300
	Leu His GIn Asp Trp Le 310 31	
Lys Cys Lys Val Ser /	Asn Lys Gly Leu Pro Se	er Ser IIe Glu Lys Thr
325	330	335
lle Ser Lys Ala Lys (Gly Gln Pro Arg Glu Pr	ro GIn Val Cys Thr Leu
340	345	350
Pro Pro Ser Gln Glu (Glu Met Thr Lys Asn Gl	n Val Ser Leu Ser Cys
355	360	365
Ala Val Lys Gly Phe ⁻	Tyr Pro Ser Asp IIe Al	a Val Glu Trp Glu Ser
370	375	380
	Asn Asn Tyr Lys Thr Th 390 39	
Ser Asp Gly Ser Phe F	Phe Leu Val Ser Arg Le	eu Thr Val Asp Lys Ser
405	410	415
Arg Trp Gln Glu Gly A	Asn Val Phe Ser Cys Se	er Val Met His Glu Ala
420	425	430
Leu His Asn His Tyr ⁻	Thr GIn Lys Ser Leu Se	er Leu Ser Leu
435	440	445
<210> 21 <211> 227 <212> PRT <213> Artificial		
<220> <223> light chain (l	LC) <tweak> with Q124E</tweak>	substitution
<400> 21		
GIn Val GIn Leu Val (GIn Ser Gly Ala Glu Va	al Lys Lys Pro Gly Ser
1 5	10	15
Ser Val Lys Val Ser (Cys Lys Ala Ser Gly Ph	ne Asp Phe Ser Thr Tyr
20	25	30
Tyr Met Ser Trp Val <i>I</i>	Arg GIn Ala Pro Gly GI	n Gly Leu Glu Trp Met
35	40	45
Gly Thr Val Tyr Val <i>H</i>	Arg GIn GIy Thr Thr Ty Page	

	50					55		eo	lf-s	eqI.	txt 60				
Asn 65	GI y	Arg	Val	Thr	Пе 70	Thr	AI a	Asp	GI u	Ser 75	Thr	Ser	Thr	AI a	Tyr 80
Met	GI u	Leu	Ser	Ser 85	Leu	Arg	Ser	GI u	Asp 90	Thr	AI a	Val	Tyr	Tyr 95	Cys
Ala	Arg	GI y	GI y 100	Tyr	Asn	Tyr	Asp	Asp 105	AI a	Phe	Val	lle	Trp 110	GI y	GI n
GLY	Thr	Leu 115	Val	Thr	Val	Ser	Ser 120	AI a	Ser	Val	AI a	AI a 125	Pro	Ser	Val
Phe	IIe 130	Phe	Pro	Pro	Ser	Asp 135	GI u	GI u	Leu	Lys	Ser 140	GI y	Thr	Al a	Ser
Val 145	Val	Cys	Leu	Leu	Asn 150	Asn	Phe	Tyr	Pro	Arg 155	GI u	Al a	Lys	Val	Gl n 160
Тгр	Lys	Val	Asp	Asn 165	Al a	Leu	GI n	Ser	GI y 170	Asn	Ser	GI n	GI u	Ser 175	Val
Thr	GI u	Gl n	Asp 180	Ser	Lys	Asp	Ser	Thr 185	Tyr	Ser	Leu	Ser	Ser 190	Thr	Leu
Thr	Leu	Ser 195	Lys	Al a	Asp	Tyr	GI u 200	Lys	Hi s	Lys	Val	Tyr 205	Al a	Cys	GI u
	Thr 210	Hi s	GI n	GI y	Leu	Ser 215	Ser	Pro	Val	Thr	Lys 220	Ser	Phe	Asn	Arg
GI y 225	GI u	Cys													
<210 <211 <212 <213	> 4 > F	22 146 PRT Arti1	Fi ci a	al											
<220 <223	> ł	neavy subs1			(HC)	<1 L-	-17>	wi tł	ר K14	47E s	subst	ti tuʻ	tion	and	K213D
<400	> 2	22													
GI u 1	Val	Gl n	Leu	Val 5	GI u	Ser	GI y	GI y	GI y 10	Leu	Val	GI n	Pro	GI y 15	GI y
Ser	Leu	Arg	Leu 20	Ser	Cys	Al a	Al a	Ser 25	GI y	Phe	Ser	Leu	Asp 30	Ser	Tyr
GI y	Val	His 35	Trp	Val	Arg	GI n	AI a 40	Thr		Lys je 29		Leu 45	GI u	Trp	Val

Ser Val IIe Trp Ser Asp Gly Thr Thr Thr Tyr Asn Ser Ala Leu Lys 50 55 60 50 60 Ser Arg Phe Thr IIe Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr Leu 65 70 75 80 GIn Met Asn Ser Leu Arg Ala GIy Asp Thr Ala Val Tyr Tyr Cys Ala 85 90 95 Arg Asp Thr His Tyr Arg Leu Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly 100 105 110 GIn Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser 115 120 125 Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala 130 135 140 Ala Leu Gly Cys Leu Val Glu Asp Tyr Phe Pro Glu Pro Val Thr Val 145 150 155 160 Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala 165 170 175 Val Leu Gin Ser Ser Giy Leu Tyr Ser Leu Ser Ser Val Val Thr Val 180 185 190 Pro Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His 195 200 205 Lys Pro Ser Asn Thr Lys Val Asp Asp Arg Val Glu Ser Lys Tyr Gly 210 215 220 Pro Pro Cys Pro Cys Pro Al a Pro Glu Phe Glu Gly Gly Pro Ser 225 230 235 240 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met IIe Ser Arg 245 250 255 Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro 260 265 270 Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala 275 280 285 Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val 290 295 300 Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr 305 310 315 320 Page 30

	Val	Ser 325	Asn	Lys	GI y	Leu	Pro 330	Ser	Ser	lle	GI u	Lys 335	Thr
lle Ser Lys	AI a 340	Lys	GI y	GI n	Pro	Arg 345	GI u	Pro	GI n	Val	Cys 350	Thr	Leu
Pro Pro Ser 355	GI n	GI u	GI u	Met	Thr 360	Lys	Asn	GI n	Val	Ser 365	Leu	Ser	Cys
Ala Val Lys 370	GI y	Phe	Tyr	Pro 375	Ser	Asp	lle	Al a	Val 380	GI u	Trp	GI u	Ser
Asn Gly Gln 385	Pro	GI u	Asn 390	Asn	Tyr	Lys	Thr	Thr 395	Pro	Pro	Val	Leu	Asp 400
Ser Asp Gly	Ser	Phe 405	Phe	Leu	Val	Ser	Arg 410	Leu	Thr	Val	Asp	Lys 415	Ser
Arg Trp GIn	GI u 420	GI y	Asn	Val	Phe	Ser 425	Cys	Ser	Val	Met	Hi s 430	GI u	Ala
Leu His Asn 435	Hi s	Tyr	Thr	GI n	Lys 440	Ser	Leu	Ser	Leu	Ser 445	Leu		
<210> 23 <211> 219 <212> PRT													
<213> Arti	fi ci a	l											
<220>	t cha	nin ((LC)	<1 L-	-17>	wi th	n Q12	24K s	subst	ti tu1	ti on	and	E123K
<220> <223> ligh ⁻	t cha	nin ((LC)	<1 L-	-17>	wi th	n Q12	24K s	subst	ti tu1	ti on	and	E123K
<220> <223> ligh ⁻ subs ⁻	t cha titut	nin (ion											
<220> <223> ligh subs <400> 23 Asp lle Val	t cha titut Met	in (i on Thr 5	GI n	Ser	Pro	Leu	Ser 10	Leu	Pro	Val	Thr	Pro 15	GI y
<220> <223> ligh subs <400> 23 Asp lle Val 1	t cha titut Met Ser 20	in (ion Thr 5 Ile	GI n Ser	Ser Cys	Pro Arg	Leu Ser 25	Ser 10 Ser	Leu Gl n	Pro Ser	Val Leu	Thr Val 30	Pro 15 Hi s	GI y Ser
<220> <223> light subst <400> 23 Asp IIe Val 1 Glu Pro Ala Asn Gly Asp	t cha titut Met Ser 20 Thr	in (ion Thr 5 Ile Tyr	GI n Ser Phe	Ser Cys Hi s	Pro Arg Trp 40	Leu Ser 25 Tyr	Ser 10 Ser Leu	Leu GI n GI n	Pro Ser Lys	Val Leu Pro 45	Thr Val 30 Gl y	Pro 15 Hi s Gl n	GI y Ser Ser
<220> <223> light subst <400> 23 Asp IIe Val 1 Glu Pro Ala Asn Gly Asp 35	t cha titut Met Ser 20 Thr Leu	Thr Thr Ile Tyr	GI n Ser Phe Tyr	Ser Cys Hi s Lys 55	Pro Arg Trp 40 Val	Leu Ser 25 Tyr Ser	Ser 10 Ser Leu Asn	Leu GI n GI n Arg	Pro Ser Lys Phe 60	Val Leu Pro 45 Ser	Thr Val 30 Gl y Gl y	Pro 15 His GIn Val	GI y Ser Ser Pro

				eo	lf-s	eqI.	txt				
Thr His Ala	Pro Phe 100	e Thr P	he Gly	Gl n 105	GI y	Thr	Lys	Leu	GI u 110	lle	Lys
Arg Thr Val 115	Ala Ala	ı Pro S	ier Val 120	Phe	lle	Phe	Pro	Pro 125	Ser	Asp	Lys
Lys Leu Lys 130	Ser Gly		la Ser 35	Val	Val	Cys	Leu 140	Leu	Asn	Asn	Phe
Tyr Pro Arg 145	Glu Ala	Lys V 150	'al GIn	Trp	Lys	Val 155	Asp	Asn	AI a	Leu	Gl n 160
Ser Gly Asn	Ser GLr 165		er Val	Thr	Gl u 170	Gl n	Asp	Ser	Lys	Asp 175	Ser
Thr Tyr Ser	Leu Ser 180	Ser T	hr Leu	Thr 185	Leu	Ser	Lys	Al a	Asp 190	Tyr	GI u
Lys His Lys 195	Val Tyr	·Ala C	5ys GLu 200	Val	Thr	Hi s	GI n	GI y 205	Leu	Ser	Ser
Pro Val Thr 210	Lys Ser		sn Arg 15	GI y	Gl u	Cys					
<210> 24 <211> 120 <212> PRT <213> Arti	fi ci al										
<220> <223> vari	able hea	ivy cha	in doma	ain V	/H <1	WEAK	< > 3	305-H	HC4		
<400> 24											
GIn Val GIn 1	Leu Val										
I	5	GIn S	Ger Gly	Al a	GI u 10	Val	Lys	Lys	Pro	GI y 15	Ser
Ser Val Lys	5		-		10		-	-		15	
	5 Val Ser 20	· Cys L	ys Ala	Ser 25	10 GI y	Phe	Asp	Phe	Ser 30	15 Thr	Tyr
Ser Val Lys Tyr Met Ser	5 Val Ser 20 Trp Val	Cys L Arg G Arg G	ys Ala In Ala 40	Ser 25 Pro	10 GI y GI y	Phe GI n	Asp GI y	Phe Leu 45	Ser 30 Gl u	15 Thr Trp	Tyr Met
Ser Val Lys Tyr Met Ser 35 Gly Thr Val	5 Val Ser 20 Trp Val Tyr Val	Cys L Arg G Arg G 5	ys Ala In Ala 40 In Gly 5	Ser 25 Pro Thr	10 GI y GI y Thr	Phe GI n Tyr	Asp GI y Tyr 60	Phe Leu 45 AI a	Ser 30 GI u Ser	15 Thr Trp Trp	Tyr Met Leu
Ser Val Lys Tyr Met Ser 35 Gly Thr Val 50 Asn Gly Arg	5 Val Ser 20 Trp Val Tyr Val Val Thr	Cys L Arg G Arg G 5 IIe T 70	ys Ala In Ala 40 In Gly 5	Ser 25 Pro Thr Asp	10 GI y GI y Thr GI u	Phe GI n Tyr Ser 75	Asp GI y Tyr 60 Thr	Phe Leu 45 Al a Ser	Ser 30 GI u Ser Thr	15 Thr Trp Trp	Tyr Met Leu Tyr 80

100 110 105 Gly Thr Leu Val Thr Val Ser Ser 115 120 <210> 25 <211> 111 <212> PRT Artificial <213> <220> <223> variable light chain domain VL <TWEAK>305-LC2 <400> 25 Asp IIe GIn Met Thr GIn Ser Pro Ser Ser Val Ser Ala Ser Val Gly 5 10 1 Asp Arg Val Thr IIe Thr Cys GIn Ala Ser GIn Asn IIe Tyr Ser Asn 20 25 30 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile 35 40 45 Tyr Thr Ala Ser Tyr Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly 50 55 60 50 Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr IIe Ser Ser Leu Gln Pro 65 70 75 80 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Thr Ala Tyr Tyr Asn Ser Arg 85 90 Pro Asp Thr Val Ala Phe Gly Gly Gly Thr Lys Val Glu IIe Lys 100 105 110 <210> 26 <211> 121 <212> PRT Artificial <213> <220> variable heavy chain domain VH <IL-17> HC136 <223> <400> 26 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly 1 5 10 15 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Asp Ser Tyr 20 25 30 Gly Val His Trp Val Arg Gln Ala Thr Gly Lys Gly Leu Glu Trp Val 35 45 40 Ser Val IIe Trp Ser Asp Gly Thr Thr Thr Tyr Asn Ser Ala Leu Lys Page 33

eolf-seql.txt 50 55 60 Ser Arg Phe Thr IIe Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr Leu 65 70 75 80 65 80 GIn Met Asn Ser Leu Arg Ala Gly Asp Thr Ala Val Tyr Tyr Cys Ala 85 90 95 Arg Asp Thr His Tyr Arg Leu Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly 100 105 110 GIn GIy Thr Thr Val Thr Val Ser Ser 115 120 <210> 27 <211> 112 <212> PRT Artificial <213> <220> <223> variable light chain domain VL <IL-17> LC136 <400> 27 Asp IIe Val Met Thr GIn Ser Pro Leu Ser Leu Pro Val Thr Pro GIy 5 10 15 Glu Pro Ala Ser IIe Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser 20 25 30 20 30 Asn Gly Asp Thr Tyr Phe His Trp Tyr Leu Gln Lys Pro Gly Gln Ser $\begin{array}{c} \mbox{Asn}\\ 35 \end{array}$ Pro GIn Leu Leu IIe Tyr Lys Val Ser Asn Arg Phe Ser GIy Val Pro 50 60 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys IIe 5 70 75 80 65 80 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Thr 85 90 95 Thr His Ala Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu IIe Lys 100 105 110 <210> 28 <211> 440 <212> PRT <213> Artificial Sequence <220> heavy chain (HC) <TWEAK> with VH-VL exchange wild type (wt) (comprising terminal GK dipeptide) <223> <400> 28

eolf-seql.txt Asp IIe GIn Met Thr GIn Ser Pro Ser Ser Val Ser Ala Ser Val Gly 1 5 10 15 Asp Arg Val Thr IIe Thr Cys GIn Ala Ser GIn Asn IIe Tyr Ser Asn 20 25 30 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu IIe 35 40 45 Tyr Thr Ala Ser Tyr Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly 50 55 60 Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr IIe Ser Ser Leu Gln Pro 65 70 75 80 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Thr Ala Tyr Tyr Asn Ser Arg 85 90 95 Pro Asp Thr Val Ala Phe Gly Gly Gly Thr Lys Val Glu IIe Lys Ser 100 105 110 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser 115 120 125 Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp 130 135 140 Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr 145 150 155 160 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr 175 165 170 Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys 180 185 190 Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp 200 205 Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala 210 215 220 Pro Glu Phe Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro 230 235 240 Lys Asp Thr Leu Met IIe Ser Arg Thr Pro Glu Val Thr Cys Val Val 245 250 Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val 260 265 270

eolf-seql.txt Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln 275 280 285 280 Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln 295 290 300 Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly 305 310 315 320 320 Leu Pro Ser Ser IIe Glu Lys Thr IIe Ser Lys Ala Lys Gly Gln Pro 325 330 335 Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Gln Glu Glu Met Thr 345 340 350 Lys Asn GIn Val Ser Leu Trp Cys Leu Val Lys GIy Phe Tyr Pro Ser 355 360 365 365 355 Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr 370 375 380 Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr 385 390 395 400 385 390 400 Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe 405 410 415 405 Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys 420 425 430 Ser Leu Ser Leu Ser Leu Gly Lys 435 440 <210> 29 <211> 448 <212> PRT <213> Artificial Sequence <220> <223> heavy chain (HC) <IL-17> with K147E substitution and K213E substitution (comprising terminal GK dipeptide) <400> 29 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly 1 5 10 15 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Asp Ser Tyr 25 20 30 Gly Val His Trp Val Arg Gln Ala Thr Gly Lys Gly Leu Glu Trp Val 35 45 40 Ser Val IIe Trp Ser Asp Gly Thr Thr Thr Tyr Asn Ser Ala Leu Lys Page 36

	50					55		eo	lf-s	eqI.	txt 60				
Ser 65	Arg	Phe	Thr	lle	Ser 70	Arg	GI u	Asn	Al a	Lys 75	Asn	Ser	Leu	Tyr	Leu 80
GI n	Met	Asn	Ser	Leu 85	Arg	AI a	GI y	Asp	Thr 90	AI a	Val	Tyr	Tyr	Cys 95	Al a
Arg	Asp	Thr	Hi s 100	Tyr	Arg	Leu	Tyr	Tyr 105	Tyr	AI a	Met	Asp	Tyr 110	Тгр	GI y
GI n	GI y	Thr 115	Thr	Val	Thr	Val	Ser 120	Ser	AI a	Ser	Thr	Lys 125	GI y	Pro	Ser
Val	Phe 130	Pro	Leu	Al a	Pro	Cys 135	Ser	Arg	Ser	Thr	Ser 140	GI u	Ser	Thr	Al a
AI a 145	Leu	GI y	Cys	Leu	Val 150	GI u	Asp	Tyr	Phe	Pro 155	GI u	Pro	Val	Thr	Val 160
Ser	Trp	Asn	Ser	GI y 165	AI a	Leu	Thr	Ser	GI y 170	Val	Hi s	Thr	Phe	Pro 175	Ala
Val	Leu	GI n	Ser 180	Ser	GI y	Leu	Tyr	Ser 185	Leu	Ser	Ser	Val	Val 190	Thr	Val
Pro	Ser	Ser 195	Ser	Leu	GI y	Thr	Lys 200	Thr	Tyr	Thr	Cys	Asn 205	Val	Asp	Hi s
Lys	Pro 210	Ser	Asn	Thr	Lys	Val 215	Asp	GI u	Arg	Val	GI u 220	Ser	Lys	Tyr	GI y
Pro 225	Pro	Cys	Pro	Pro	Cys 230	Pro	Al a	Pro	GI u	Phe 235	GI u	GI y	GI y	Pro	Ser 240
Val	Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp 250	Thr	Leu	Met	lle	Ser 255	Arg
Thr	Pro	GI u	Val 260	Thr	Cys	Val	Val	Val 265	Asp	Val	Ser	GI n	GI u 270	Asp	Pro
GI u	Val	Gl n 275	Phe	Asn	Trp	Tyr	Val 280	Asp	GI y	Val	GI u	Val 285	Hi s	Asn	Al a
Lys	Thr 290	Lys	Pro	Arg	GI u	GI u 295	GI n	Phe	Asn	Ser	Thr 300	Tyr	Arg	Val	Val
Ser 305	Val	Leu	Thr	Val	Leu 310	Hi s	GI n	Asp	Trp	Leu 315	Asn	GI y	Lys	GI u	Tyr 320
Lys	Cys	Lys	Val	Ser	Asn	Lys	GI y	Leu		Ser Je 37		lle	GI u	Lys	Thr

								eo	l f-s	eal.	txt				
				325					330					335	
lle	Ser	Lys	AI a 340	Lys	GI y	GI n	Pro	Arg 345	GI u	Pro	GI n	Val	Cys 350	Thr	Leu
Pro	Pro	Ser 355	GI n	GI u	GI u	Met	Thr 360	Lys	Asn	GI n	Val	Ser 365	Leu	Ser	Cys
AI a	Val 370	Lys	GI y	Phe	Tyr	Pro 375	Ser	Asp	lle	AI a	Val 380	GI u	Trp	GI u	Ser
Asn 385	GI y	GI n	Pro	GI u	Asn 390	Asn	Tyr	Lys	Thr	Thr 395	Pro	Pro	Val	Leu	Asp 400
Ser	Asp	GI y	Ser	Phe 405	Phe	Leu	Val	Ser	Arg 410	Leu	Thr	Val	Asp	Lys 415	Ser
Arg	Тгр	GI n	GI u 420	GI y	Asn	Val	Phe	Ser 425	Cys	Ser	Val	Met	Hi s 430	GI u	Al a
Leu	Hi s	Asn 435	Hi s	Tyr	Thr	GI n	Lys 440	Ser	Leu	Ser	Leu	Ser 445	Leu	GI y	Lys
<21 <21 <21	1> 4	30 448 PRT													
<21	3> 1	Artii	ficia	al Se	equer	nce									
<21 <22 <22	0> 3> ł		y cha	ain	(HC)	<1 L-								and	K213D
<22 <22 <40	0> 3> H 9	neavy subsi 30	y cha ti tu†	ain tion	(HC) (cor	<ll npris</ll 	si ng	terr	ni nal	GK	di p€	eptio	de)		
<22 <22 <40	0> 3> s	neavy subsi 30	y cha ti tu†	ain tion	(HC) (cor	<ll npris</ll 	si ng	terr	ni nal	GK	di p€	eptio	de)		
<22 <22 <40 GI u 1	0> 3> H 9	neavy subsi 30 GI n	y cha ti tut Leu	ain tion Val 5	(HC) (cor GI u	<ll mpris</ll 	si ng GI y	terr GI y	ni nal Gl y 10	GK Leu	di pe Val	eptio GIn	de) Pro	GI y 15	GI y
<22 <22 <40 GI u 1 Ser	0> 3> 9 0> 3 Val	neavy subsi 30 GI n Arg	y cha ti tu Leu Leu 20	val 5 Ser	(HC) (cor GI u Cys	<ll>SerAla</ll>	GI y AI a	terr Gly Ser 25	GI y 10 GI y	GK Leu Phe	di pe Val Ser	GI n Leu	de) Pro Asp 30	GI y 15 Ser	GI y Tyr
<22 <22 <40 GI u 1 Ser GI y	0> 3> 4 9 0> 3 Val Leu	neavy subs 30 GI n Arg Hi s 35	y cha ti tu Leu 20 Trp	Val 5 Val 5 Val	(HC) (cor GI u Cys Arg	<ll>SerAl aGl n</ll>	GI y AI a AI a 40	terr Gly Ser 25 Thr	GI y 10 GI y GI y	GK Leu Phe Lys	di pe Val Ser Gl y	GI n Leu Leu 45	de) Pro Asp 30 Gl u	GI y 15 Ser Trp	GI y Tyr Val
<22 <22 <40 GI u 1 Ser GI y Ser	0> 3> 4 9 0> 3 Val Leu Val	neavy subsi 30 GI n Arg Hi s 35 II e	y cha ti tu Leu 20 Trp Trp	val 5 Ser Val Ser	(HC) (cor GI u Cys Arg Asp	Ser Al a Gl n Gl y 55	GI y AI a AI a 40 Thr	terr Gly Ser 25 Thr Thr	ni nal GI y 10 GI y GI y Thr	GK Leu Phe Lys Tyr	di pe Val Ser Gl y Asn 60	GIn Leu Leu 45 Ser	de) Pro Asp 30 GI u AI a	GI y 15 Ser Trp Leu	GI y Tyr Val Lys
<22 <22 <40 GI u 1 Ser GI y Ser 5	0> 3> 1 2 Val Leu Val Val 50	neavy subsi 30 GI n Arg Hi s 35 II e Phe	y cha ti tui Leu Leu 20 Trp Trp Trp	Val 5 Ser Val Ser IIe	(HC) (cor GI u Cys Arg Asp Ser 70	SerAl aGl nGl y55Arg	GI y AI a AI a 40 Thr GI u	terr GI y Ser 25 Thr Thr Asn	ni nal GI y TO GI y Thr AI a	GK Leu Phe Lys Tyr Lys 75	di pe Val Ser Gl y Asn 60 Asn	GI n Leu Leu 45 Ser Ser	de) Pro Asp 30 GI u AI a Leu	GI y 15 Ser Trp Leu Tyr	GI y Tyr Val Lys Leu 80

GI n	GI y	Thr 115	Thr	Val	Thr	Val	Ser 120	Ser	Al a	Ser	Thr	Lys 125	GI y	Pro	Ser
Val	Phe 130	Pro	Leu	Ala	Pro	Cys 135	Ser	Arg	Ser	Thr	Ser 140	GI u	Ser	Thr	Al a
AI a 145	Leu	GI y	Cys	Leu	Val 150	GI u	Asp	Tyr	Phe	Pro 155	GI u	Pro	Val	Thr	Val 160
Ser	Trp	Asn	Ser	GI y 165	Al a	Leu	Thr	Ser	GI y 170	Val	Hi s	Thr	Phe	Pro 175	Al a
Val	Leu	GI n	Ser 180	Ser	GI y	Leu	Tyr	Ser 185	Leu	Ser	Ser	Val	Val 190	Thr	Val
Pro	Ser	Ser 195	Ser	Leu	GI y	Thr	Lys 200	Thr	Tyr	Thr	Cys	Asn 205	Val	Asp	Hi s
Lys	Pro 210	Ser	Asn	Thr	Lys	Val 215	Asp	Asp	Arg	Val	GI u 220	Ser	Lys	Tyr	GI y
Pro 225	Pro	Cys	Pro	Pro	Cys 230	Pro	Al a	Pro	GI u	Phe 235	GI u	GI y	GI y	Pro	Ser 240
Val	Phe	Leu	Phe	Pro 245	Pro	Lys	Pro	Lys	Asp 250	Thr	Leu	Met	lle	Ser 255	Arg
Thr	Pro	GI u	Val 260	Thr	Cys	Val	Val	Val 265	Asp	Val	Ser	GI n	GI u 270	Asp	Pro
GI u	Val	Gl n 275	Phe	Asn	Trp	Tyr	Val 280	Asp	GI y	Val	GI u	Val 285	Hi s	Asn	Al a
Lys	Thr 290	Lys	Pro	Arg	GI u	GI u 295	Gl n	Phe	Asn	Ser	Thr 300	Tyr	Arg	Val	Val
Ser 305	Val	Leu	Thr	Val	Leu 310	Hi s	Gl n	Asp	Trp	Leu 315	Asn	GI y	Lys	GI u	Tyr 320
Lys	Cys	Lys	Val	Ser 325	Asn	Lys	GI y	Leu	Pro 330	Ser	Ser	lle	GI u	Lys 335	Thr
lle	Ser	Lys	AI a 340	Lys	GI y	GI n	Pro	Arg 345	GI u	Pro	Gl n	Val	Cys 350	Thr	Leu
Pro	Pro	Ser 355	Gl n	GI u	GI u	Met	Thr 360	Lys	Asn	Gl n	Val	Ser 365	Leu	Ser	Cys
AI a	Val 370	Lys	GI y	Phe	Tyr	Pro 375	Ser	Asp	_	Ala e 39	380	GI u	Trp	GI u	Ser

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp 390 395 385 400 Ser Asp Gly Ser Phe Phe Leu Val Ser Arg Leu Thr Val Asp Lys Ser 41Ŏ 405 415 Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala 420 425 430 Leu His Asn His Tyr Thr GIn Lys Ser Leu Ser Leu Ser Leu GIy Lys 435 440 445 <210> 31 459 <211> <212> PRT Artificial Sequence <213> <220> heavy chain (HC) <Ang-2> wild type (wt) (comprising terminal GK <223> di pepti de) <400> 31 GIn Val GIn Leu Val GIu Ser GIy Ala GIu Val Lys Lys Pro GIy Ala 1 5 10 15 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr 20 25 30 20 Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met 35 40 45 35 Gly Trp IIe Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe 50 60 GIn GIy Arg Val Thr Met Thr Arg Asp Thr Ser IIe Ser Thr Ala Tyr 65 70 75 80 Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys 85 90 95 Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr 100 105 110 100 Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser 115 120 125 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser 135 130 140 Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp 145 150 155 160

								eo	lf-s	eqI.	txt				
Tyr	Phe	Pro	GI u	Pro 165	Val	Thr	Val	Ser	Trp 170	Asn	Ser	GI y	Al a	Leu 175	Thr
Ser	GI y	Val	Hi s 180	Thr	Phe	Pro	Al a	Val 185	Leu	GI n	Ser	Ser	GI y 190	Leu	Tyr
Ser	Leu	Ser 195	Ser	Val	Val	Thr	Val 200	Pro	Ser	Ser	Ser	Leu 205	GI y	Thr	GI n
Thr	Tyr 210	lle	Cys	Asn	Val	Asn 215	Hi s	Lys	Pro	Ser	Asn 220	Thr	Lys	Val	Asp
Lys 225	Lys	Val	GI u	Pro	Lys 230	Ser	Cys	Asp	Lys	Thr 235	Hi s	Thr	Cys	Pro	Pro 240
Cys	Pro	Al a	Pro	GI u 245	Leu	Leu	GI y	GI y	Pro 250	Ser	Val	Phe	Leu	Phe 255	Pro
Pro	Lys	Pro	Lys 260	Asp	Thr	Leu	Met	IIе 265	Ser	Arg	Thr	Pro	GI u 270	Val	Thr
Cys	Val	Val 275	Val	Asp	Val	Ser	Hi s 280	GI u	Asp	Pro	GI u	Val 285	Lys	Phe	Asn
Тгр	Tyr 290	Val	Asp	GI y	Val	GI u 295	Val	Hi s	Asn	Al a	Lys 300	Thr	Lys	Pro	Arg
GI u 305	GI u	GI n	Tyr	Asn	Ser 310	Thr	Tyr	Arg	Val	Val 315	Ser	Val	Leu	Thr	Val 320
Leu	Hi s	GI n	Asp	Trp 325	Leu	Asn	GI y	Lys	GI u 330	Tyr	Lys	Cys	Lys	Val 335	Ser
Asn	Lys	Al a	Leu 340	Pro	Al a	Pro	lle	GI u 345	Lys	Thr	lle	Ser	Lys 350	Al a	Lys
GI y	GI n	Pro 355	Arg	GI u	Pro	GI n	Val 360	Tyr	Thr	Leu	Pro	Pro 365	Cys	Arg	Asp
GI u	Leu 370	Thr	Lys	Asn	GI n	Val 375	Ser	Leu	Trp	Cys	Leu 380	Val	Lys	GI y	Phe
Tyr 385	Pro	Ser	Asp	lle	AI a 390	Val	GI u	Trp	GI u	Ser 395	Asn	GI y	GI n	Pro	GI u 400
Asn	Asn	Tyr	Lys	Thr 405	Thr	Pro	Pro	Val	Leu 410	Asp	Ser	Asp	GI y	Ser 415	Phe
Phe	Leu	Tyr	Ser 420	Lys	Leu	Thr	Val	Asp 425	Lys	Ser	Arg	Trp	GI n 430	GI n	GI y

eolf-seql.txt Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr 435 440 445 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys 450 455 <210> 32 439 <211> <212> PRT <213> Artificial Sequence <220> <223> heavy chain (HC) <VEGF> with VH-VL exchange wild type (wt) (comprising terminal GK dipeptide) <400> 32 Asp IIe GIn Met Thr GIn Ser Pro Ser Ser Leu Ser Ala Ser Val Gly 1 5 10 15 Asp Arg Val Thr IIe Thr Cys Ser Ala Ser Gln Asp IIe Ser Asn Tyr 20 25 30 Leu Asn Trp Tyr GIn GIn Lys Pro GIy Lys Ala Pro Lys Val Leu IIe 35 40 45 Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly 50 55 60 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr IIe Ser Ser Leu Gln Pro 65 70 75 80 65 80 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp 85 90 Thr Phe Gly Gln Gly Thr Lys Val Glu IIe Lys Ser Ser Ala Ser Thr 100 105 110 Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser 115 120 125 Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu 130 135 140 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His 145 150 155 160 Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser 165 170 175 165 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr IIe Cys 180 185 190

eolf-seql.txt Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu 200 205 Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro 210 215 220 Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met IIe 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 260 265 270 Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr 275 280 285 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 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg 325 330 335 Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn GIn Val Ser Leu Ser Cys Ala Val Lys GIy Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys 370 375 380 Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val 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 His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys <210> <211> <212> PRT

<213> Artificial Sequence

<220> <223> heavy chain (HC) <ang-2> with K147E substitution (comprising terminal GK dipeptide)</ang-2>
<400> 33
GIn Val GIn Leu Val GIu Ser GIy Ala GIu Val Lys Lys Pro GIy Ala 1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr 20 25 30
Tyr Met His Trp Val Arg GIn Ala Pro Gly GIn Gly Leu Glu Trp Met 35 40 45
Gly Trp IIe Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe 50 55 60
GIn GIy Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr 65 70 75 80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys 85 90 95
Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr 100 105 110
Pro Gly Ala Phe Asp IIe Trp Gly Gln Gly Thr Met Val Thr Val Ser 115 120 125
Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser 130 135 140
Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Glu Asp 145 150 155 160
Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr 165 170 175
Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr 180 185 190
Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln 195 200 205
Thr Tyr IIe Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp 210 215 220
Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro 225 230 235 240
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Page 44

				245				ec	l f-s 250	eqI.	txt			255	
Pro	Lys	Pro	Lys 260	Asp	Thr	Leu	Met	IIе 265	Ser	Arg	Thr	Pro	GI u 270	Val	Thr
Cys	Val	Val 275	Val	Asp	Val	Ser	Hi s 280	GI u	Asp	Pro	GI u	Val 285	Lys	Phe	Asn
Тгр	Tyr 290	Val	Asp	GI y	Val	GI u 295	Val	Hi s	Asn	AI a	Lys 300	Thr	Lys	Pro	Arg
GI u 305	GI u	GI n	Tyr	Asn	Ser 310	Thr	Tyr	Arg	Val	Val 315	Ser	Val	Leu	Thr	Val 320
Leu	Hi s	GI n	Asp	Trp 325	Leu	Asn	GI y	Lys	GI u 330	Tyr	Lys	Cys	Lys	Val 335	Ser
Asn	Lys	Al a	Leu 340	Pro	Al a	Pro	lle	GI u 345	Lys	Thr	lle	Ser	Lys 350	AI a	Lys
GI y	GI n	Pro 355	Arg	GI u	Pro	GI n	Val 360	Tyr	Thr	Leu	Pro	Pro 365	Cys	Arg	Asp
GI u	Leu 370	Thr	Lys	Asn	GI n	Val 375	Ser	Leu	Trp	Cys	Leu 380	Val	Lys	GI y	Phe
Tyr 385	Pro	Ser	Asp	lle	AI a 390	Val	GI u	Trp	GI u	Ser 395	Asn	GI y	GI n	Pro	GI u 400
Asn	Asn	Tyr	Lys	Thr 405	Thr	Pro	Pro	Val	Leu 410	Asp	Ser	Asp	GI y	Ser 415	Phe
Phe	Leu	Tyr	Ser 420	Lys	Leu	Thr	Val	Asp 425	Lys	Ser	Arg	Trp	GI n 430	GI n	GI y
Asn	Val	Phe 435	Ser	Cys	Ser	Val	Met 440	Hi s	GI u	Al a	Leu	Hi s 445	Asn	Hi s	Tyr
Thr	GI n 450	Lys	Ser	Leu	Ser	Leu 455	Ser	Pro	GI y	Lys					
<210 <211 <212 <213	> 4 2>	34 459 PRT Arti1	ficia	al Se	equei	nce									
<220 <223	8> 1			ain GK d				wi tł	K2´	13E s	subst	ti tu	ti on	(cor	nprising
<400)> :	34													
Gl n 1	Val	GI n	Leu	Val 5	GI u	Ser	GI y	AI a	10	Val je 45	-	Lys	Pro	GI y 15	Al a

Ser Val L	ys Val 20	Ser C	Sys Lys	Al a	Ser 25	GI y	Tyr	Thr	Phe	Thr 30	GI y	Tyr
Tyr Met H 3	lis Trp 5	Val A	vrg Gln	Al a 40	Pro	GI y	Gl n	GI y	Leu 45	GI u	Trp	Met
Gly Trp I 50	le Asn	Pro A	sn Ser 55	GI y	GI y	Thr	Asn	Tyr 60	Al a	GI n	Lys	Phe
GIn GIy A 65	rg Val		let Thr '0	Arg	Asp	Thr	Ser 75	lle	Ser	Thr	Al a	Tyr 80
Met Glu L	eu Ser	Arg L 85	.eu Arg	Ser	Asp	Asp 90	Thr	Al a	Val	Tyr	Tyr 95	Cys
Ala Arg S	Ser Pro 100	Asn P	Pro Tyr	Tyr	Tyr 105	Asp	Ser	Ser	GI y	Tyr 110	Tyr	Tyr
Pro Gly A 1	la Phe 15	Asp I	le Trp	GI y 120	GI n	GI y	Thr	Met	Val 125	Thr	Val	Ser
Ser Ala S 130	Ser Thr	Lys G	ly Pro 135		Val	Phe	Pro	Leu 140	Al a	Pro	Ser	Ser
Lys Ser T 145	hr Ser		ily Thr 50	Al a	Al a	Leu	GI y 155	Cys	Leu	Val	Lys	Asp 160
Tyr Phe P	Pro Glu	Pro V 165	'al Thr	Val	Ser	Trp 170	Asn	Ser	GI y	Al a	Leu 175	Thr
Ser Gly V	/al His 180	Thr P	Phe Pro	Al a	Val 185	Leu	Gl n	Ser	Ser	GI y 190	Leu	Tyr
Ser Leu S 1	ser Ser 95	Val V	'al Thr	Val 200	Pro	Ser	Ser	Ser	Leu 205	GI y	Thr	GI n
Thr Tyr I 210	le Cys	Asn V	al Asn 215		Lys	Pro	Ser	Asn 220	Thr	Lys	Val	Asp
Glu Lys V 225	/al Glu		ys Ser 30	Cys	Asp	Lys	Thr 235	Hi s	Thr	Cys	Pro	Pro 240
Cys Pro A	la Pro	Glu L 245	.eu Leu	GI y	GI y	Pro 250	Ser	Val	Phe	Leu	Phe 255	Pro
Pro Lys P	ro Lys 260	Asp T	ħr Leu	Met	Пе 265	Ser	Arg	Thr	Pro	GI u 270	Val	Thr
	Val Val 275	Asp V	'al Ser	Hi s 280	GI u		Pro e 46		Val 285	Lys	Phe	Asn

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg 290 295 300
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val 305 310 315 320
Leu His GIn Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser 325 330 335
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys 340 345 350
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp 355 360 365
Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe 370 375 380
Tyr Pro Ser Asp IIe Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu 385 390 395 400
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe 405 410 415
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly 420 425 430
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr 435 440 445
Thr GIn Lys Ser Leu Ser Leu Ser Pro GIy Lys 450 455
<210> 35 <211> 459 <212> PRT <213> Artificial Sequence
<220> <223> heavy chain (HC) <ang-2> with K147E substitution and K213E substitution (comprising terminal GK dipeptide)</ang-2>
<400> 35
GIn Val GIn Leu Val GIu Ser GIy Ala GIu Val Lys Lys Pro GIy Ala 1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr 20 25 30
Tyr Met His Trp Val Arg GIn Ala Pro Gly GIn Gly Leu Glu Trp Met 35 40 45

		ec	olf-seql.	txt		
Gly Trp lle As 50	n Pro Asn Se 55	r Gly Gly	Thr Asn	Tyr Ala 60	GIn Lys	Phe
Gln Gly Arg Va 65	Thr Met Th 70	r Arg Asp	Thr Ser 75	lle Ser	Thr Ala	Tyr 80
Met Glu Leu Se	r Arg Leu Ar 85	g Ser Asp	Asp Thr 90	Ala Val	Tyr Tyr 95	Cys
Ala Arg Ser Pr 10		r Tyr Tyr 105	Asp Ser	Ser Gly	Tyr Tyr 110	Tyr
Pro Gly Ala Ph 115	e Asp lle Tr	pGlyGln 120	Gly Thr	Met Val 125	Thr Val	Ser
Ser Ala Ser Th 130	r Lys Gly Pr 13		Phe Pro	Leu Ala 140	Pro Ser	Ser
Lys Ser Thr Se 145	r Gly Gly Th 150	r Ala Ala	Leu Gly 155	Cys Leu	Val Glu	Asp 160
Tyr Phe Pro Gl	u Pro Val Th 165	r Val Ser	Trp Asn 170	Ser Gly	Ala Leu 175	Thr
Ser Gly Val Hi 18	s Thr Phe Pr)	o Ala Val 185	Leu GIn	Ser Ser	GLy Leu 190	Tyr
Ser Leu Ser Se 195	r Val Val Th	r Val Pro 200	Ser Ser	Ser Leu 205	Gly Thr	GI n
Thr Tyr IIe Cy 210	s Asn Val As 21		Pro Ser	Asn Thr 220	Lys Val	Asp
GLu Lys Val Gl 225	u Pro Lys Se 230	r Cys Asp	Lys Thr 235	His Thr	Cys Pro	Pro 240
Cys Pro Ala Pr	o GLu Leu Le 245	u Gly Gly	Pro Ser 250	Val Phe	Leu Phe 255	Pro
Pro Lys Pro Ly 26		u Met IIe 265	Ser Arg	Thr Pro	GLu Val 270	Thr
Cys Val Val Va 275	Asp Val Se	r His Glu 280	Asp Pro	GLu Val 285	Lys Phe	Asn
Trp Tyr Val As 290	o Gly Val Gl 29		Asn Ala	Lys Thr 300	Lys Pro	Arg
Glu Glu Gln Ty 305	r Asn Ser Th 310	r Tyr Arg	Val Val 315	Ser Val	Leu Thr	Val 320

							eo	lf-s	eqI.	txt				
Leu Hi	s GIn	Asp	Trp 325	Leu	Asn	GI y	Lys	GI u 330	Tyr	Lys	Cys	Lys	Val 335	Ser
Asn Ly	s Ala	Leu 340	Pro	Al a	Pro	lle	GI u 345	Lys	Thr	lle	Ser	Lys 350	Al a	Lys
GI y GI	n Pro 355	Arg	GI u	Pro	GI n	Val 360	Tyr	Thr	Leu	Pro	Pro 365	Cys	Arg	Asp
GLU Le 37		Lys	Asn	GI n	Val 375	Ser	Leu	Trp	Cys	Leu 380	Val	Lys	GI y	Phe
Tyr Pr 385	o Ser	Asp	lle	AI a 390	Val	GI u	Trp	GI u	Ser 395	Asn	GI y	GI n	Pro	GI u 400
Asn As	n Tyr	Lys	Thr 405	Thr	Pro	Pro	Val	Leu 410	Asp	Ser	Asp	GI y	Ser 415	Phe
Phe Le	u Tyr	Ser 420	Lys	Leu	Thr	Val	Asp 425	Lys	Ser	Arg	Trp	GI n 430	GI n	GI y
Asn Va	I Phe 435	Ser	Cys	Ser	Val	Met 440	Hi s	GI u	AI a	Leu	Hi s 445	Asn	Hi s	Tyr
Thr GI 45		Ser	Leu	Ser	Leu 455	Ser	Pro	GI y	Lys					
<210> <211> <212> <213>	36 459 PRT Arti	ficia	al Se	equer	nce									
<220> <223>	heav subs												and	K213D
<400>	36													
GIn Va 1	I GIn	Leu	Val 5	GI u	Ser	GI y	Al a	GI u 10	Val	Lys	Lys	Pro	GI y 15	Ala
Ser Va	l Lys	Val 20	Ser	Cys	Lys	Al a	Ser 25	GI y	Tyr	Thr	Phe	Thr 30	GI y	Tyr
Tyr Me	tHis 35	Тгр	Val	Arg	GI n	Al a 40	Pro	GI y	GI n	GI y	Leu 45	GI u	Trp	Met
GIy Tr 50		Asn	Pro	Asn	Ser 55	GI y	GI y	Thr	Asn	Tyr 60	AI a	GI n	Lys	Phe
GIn GI 65	y Arg	Val	Thr	Met 70	Thr	Arg	Asp	Thr	Ser 75	lle	Ser	Thr	Al a	Tyr 80

eolf-seql.txt Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys 85 90 95 Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr 100 105 110 100 Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser 115 120 125 125 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser 130 135 140 Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Glu Asp 145 150 155 160 Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr 165 170 175 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr 185 190 180 Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln 200 195 205 Thr Tyr IIe Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp 210 215 220 Asp Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro 225 230 235 240 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro 245 250 250 255 245 Pro Lys Pro Lys Asp Thr Leu Met IIe Ser Arg Thr Pro Glu Val Thr 260 265 270 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn 275 280 285 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg 290 295 300 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val 305 310 315 320 Leu His GIn Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser 325 330 335 Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys 340 345 350

eolf-seql.txt Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp 355 360 365 Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe 375 370 380 Tyr Pro Ser Asp IIe 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 405 410 415 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly 420 425 430 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr 435 440 445 Thr GIn Lys Ser Leu Ser Leu Ser Pro GIy Lys 450 455 <210> 37 <211> 448 <212> PRT Artificial Sequence <213> <220> heavy chain (HC) <IL-17> wild type (wt) (comprising terminal GK <223> dipeptide) <400> 37 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly 1 5 10 15 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Asp Ser Tyr 20 25 30 Gly Val His Trp Val Arg Gln Ala Thr Gly Lys Gly Leu Glu Trp Val 35 40 45 Ser Val IIe Trp Ser Asp Gly Thr Thr Thr Tyr Asn Ser Ala Leu Lys 50 55 60 50 60 Ser Arg Phe Thr IIe Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr Leu 65 70 75 80 80 GIn Met Asn Ser Leu Arg Ala GIy Asp Thr Ala Val Tyr Tyr Cys Ala 90 85 Arg Asp Thr His Tyr Arg Leu Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly 100 105 110 GIn GIy Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys GIy Pro Ser Page 51

115		120	eol f-sec		125	
Val Phe Pro 130	Leu Ala Pro	Cys Ser 135	Arg Ser T	hr Ser 140	Glu Ser	Thr Ala
Ala Leu Gly 145	Cys Leu Val 150	Lys Asp		ro Glu 55	Pro Val	Thr Val 160
Ser Trp Asn	Ser GLy Ala 165	Leu Thr	Ser Gly Va 170	al His	Thr Phe	Pro Ala 175
Val Leu Gln	Ser Ser Gly 180	Leu Tyr	Ser Leu So 185	er Ser	Val Val 190	Thr Val
Pro Ser Ser 195	Ser Leu Gly	Thr Lys 200	Thr Tyr T		Asn Val 205	Asp His
Lys Pro Ser 210	Asn Thr Lys	Val Asp 215	Lys Arg V	al Glu 220	Ser Lys	Tyr Gly
Pro Pro Cys 225	Pro Pro Cys 230	Pro Ala		he Glu 35	Gly Gly	Pro Ser 240
Val Phe Leu	Phe Pro Pro 245	Lys Pro	Lys Asp T 250	hr Leu	Met lle	Ser Arg 255
Thr Pro Glu	Val Thr Cys 260		Val Asp Va 265	al Ser	GIn GIu 270	Asp Pro
Glu Val Gln 275	Phe Asn Trp	Tyr Val 280	Asp Gly Va		Val His 285	Asn Ala
Lys Thr Lys 290	Pro Arg Glu	Glu Gln 295	Phe Asn S	er Thr 300	Tyr Arg	Val Val
Ser Val Leu 305	Thr Val Leu 310	His GIn		eu Asn 15	GIy Lys	Glu Tyr 320
Lys Cys Lys	Val Ser Asn 325	Lys Gly	Leu Pro So 330	er Ser	lle Glu	Lys Thr 335
lle Ser Lys	Ala Lys Gly 340	GIn Pro	Arg Glu P 345	ro GIn	Val Cys 350	Thr Leu
Pro Pro Ser 355	Gln Glu Glu	Met Thr 360	Lys Asn G		Ser Leu 365	Ser Cys
ALa Val Lys 370	Gly Phe Tyr	Pro Ser 375	Asp lle A	La Val 380	Glu Trp	Glu Ser
Asn Gly Gln	Pro Glu Asn	Asn Tyr	Lys Thr T Page		Pro Val	Leu Asp

385	eolf-seql.txt 390 395	400
Ser Asp Gly Ser Phe 405	Phe Leu Val Ser Arg Leu Thr Val Asp Lys 410 415	
Arg Trp Gln Glu Gly 420	Asn Val Phe Ser Cys Ser Val Met His Glu 425 430	ıAla
Leu His Asn His Tyr 435	Thr GIn Lys Ser Leu Ser Leu Gly 440 445	/ Lys
<210> 38 <211> 688 <212> PRT <213> Artificial Se	equence	
<ang-2> wild</ang-2>	b heavy chain (HC) including two heavy o type (wt) coupled to one heavy chain (H omain exchange wild type (wt) via ne-linkers	
<400> 38		
GIn Val GIn Leu Val 1 5	Glu Ser Gly Ala Glu Val Lys Lys Pro Gly 10 15	/ Ala
Ser Val Lys Val Ser 20	Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly 25 30	ı Tyr
Tyr Met His Trp Val 35	Arg GIn Ala Pro Gly GIn Gly Leu Glu Trp 40 45	o Met
Gly Trp Ile Asn Pro 50	Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys 55 60	s Phe
GIn GIy Arg Val Thr 65	Met Thr Arg Asp Thr Ser Ile Ser Thr Ala 70 75	a Tyr 80
Met Glu Leu Ser Arg 85	Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr 90 95	- Cys
Ala Arg Ser Pro Asn 100	Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr 105 110	- Tyr
Pro Gly Ala Phe Asp 115	lle Trp Gly Gln Gly Thr Met Val Thr Val 120 125	Ser
Ser Ala Ser Thr Lys 130	Gly Pro Ser Val Phe Pro Leu Ala Pro Ser 135 140	Ser
Lys Ser Thr Ser Gly 145	Gly Thr Ala Ala Leu Gly Cys Leu Val Lys 150 155	6 Asp 160

eolf-seql.txt Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr 165 170 175 165 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr 180 185 190 Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln 200 205 Thr Tyr IIe Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp 210 215 220 Lys Lys Val Glu Pro Lys Ser Cys Ser Gly Gly Gly Gly Ser Gln Val 225 230 235 240 GIN Leu Val GIU Ser GIY Ala GIU Val Lys Lys Pro GIY Ala Ser Val 245 250 255 Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr Tyr Met 260 265 270 His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Trp 280 285 lle Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe Gln Gly 290 295 300 Arg ValThr Met Thr Arg Asp Thr Ser IIeSer Thr Ala Tyr Met Glu305310315320 Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg 325 330 335 Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Gly Tyr Tyr Tyr Pro Gly 340 345 350 340 Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala 355 360 365 Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser 370 375 380 Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe 390 385 395 400 Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly 405 410 415 Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu 420 425 430

eolf-seql.txt Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr 435 440 445 II e Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys 450 455 460 Val Glu Pro Lys Ser Cys Gly Ser Gly Gly Gly Ser Asp Ile Gln Met 465 470 475 480 480 465 Thr GIn Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr 490 495 485 Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr 500 510 505 GIN GIN Lys Pro GIY Lys Ala Pro Lys Val Leu IIe Tyr Phe Thr Ser 515 520 525 Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly 535 54Ó 530 Thr Asp Phe Thr Leu Thr IIe Ser Ser Leu GIn Pro Glu Asp Phe Ala 545 550 555 560 Thr Tyr Tyr Cys GIn GIn Tyr Ser Thr Val Pro Trp Thr Phe GIy GIn 565 570 575 Gly Thr Lys Val Glu IIe Lys Ser Ser Ala Ser Thr Lys Gly Pro Ser 585 590 580 Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala 595 600 605 Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val 610 615 620 Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala 625 630 635 640 Val Leu Gin Ser Ser Giy Leu Tyr Ser Leu Ser Ser Val Val Thr Val 645 650 655 Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His 660 665 670 Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys 675 68Ö 685

<210> 39 <211> 688 <212> PRT

<213> Artificial Sequence

<pre><220> <223> Fab2-CrossFab heavy chain (HC) including two heavy chains (HC) <ang-2> with K147E and K213E substitutions coupled to one heavy chain (HC) <vegf> with VL-VH domain exchange wild type (wt) via glycine-serine-linkers</vegf></ang-2></pre>
<400> 39
GIn Val GIn Leu Val GIu Ser GIy Ala GIu Val Lys Lys Pro GIy Ala 1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr 20 25 30
Tyr Met His Trp Val Arg GIn Ala Pro Gly GIn Gly Leu Glu Trp Met 35 40 45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe 50 55 60
GIn GIy Arg Val Thr Met Thr Arg Asp Thr Ser IIe Ser Thr Ala Tyr 65 70 75 80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys 85 90 95
Ala Arg Ser Pro Asn Pro Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr 100 105 110
Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser 115 120 125
Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser 130 135 140
Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Glu Asp 145 150 155 160
Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr 165 170 175
Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr 180 185 190
Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln 195 200 205
Thr Tyr IIe Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp 210 215 220
Glu Lys Val Glu Pro Lys Ser Cys Ser Gly Gly Gly Gly Ser Gln Val 225 230 235 240

				eo	lf-s	eqI.	txt				
Gln Leu Val (Glu Ser 245	GIY AI	a Glu	Val	Lys 250	Lys	Pro	GI y	Al a	Ser 255	Val
Lys Val Ser (Cys Lys 260	Ala Se	er Gly	Tyr 265	Thr	Phe	Thr	GI y	Tyr 270	Tyr	Met
His Trp Val <i>H</i> 275	Arg Gln	Ala Pr	o GLy 280	GI n	GI y	Leu	GI u	Trp 285	Met	GI y	Trp
lle Asn Pro A 290	Asn Ser	GI y GI 29	y Thr 95	Asn	Tyr	Al a	GI n 300	Lys	Phe	GI n	GI y
Arg Val Thr M 305	Met Thr	Arg As 310	sp Thr	Ser	lle	Ser 315	Thr	Al a	Tyr	Met	GI u 320
Leu Ser Arg l	Leu Arg 325	Ser As	sp Asp	Thr	AI a 330	Val	Tyr	Tyr	Cys	AI a 335	Arg
Ser Pro Asn I	Pro Tyr 340	Tyr Ty	vr Asp	Ser 345	Ser	GI y	Tyr	Tyr	Tyr 350	Pro	GI y
Ala Phe Asp I 355	lle Trp	GI y GI	n Gly 360	Thr	Met	Val	Thr	Val 365	Ser	Ser	AI a
Ser Thr Lys (370	Gly Pro	Ser Va 37		Pro	Leu	Al a	Pro 380	Ser	Ser	Lys	Ser
Thr Ser Gly (385	Gly Thr	Ala Al 390	a Leu	GI y	Cys	Leu 395	Val	GI u	Asp	Tyr	Phe 400
Pro Glu Pro N	Val Thr 405	Val Se	er Trp	Asn	Ser 410	GI y	Al a	Leu	Thr	Ser 415	GI y
Val His Thr F	Phe Pro 420	Ala Va	I Leu	GI n 425	Ser	Ser	GI y	Leu	Tyr 430	Ser	Leu
Ser Ser Val N 435	Val Thr	Val Pr	o Ser 440	Ser	Ser	Leu	GI y	Thr 445	GI n	Thr	Tyr
lle Cys Asn \ 450	Val Asn	His Ly 45		Ser	Asn	Thr	Lys 460	Val	Asp	GI u	Lys
Val Glu Pro l 465	Lys Ser	Cys GI 470	y Ser	GI y	GI y	GI y 475	Ser	Asp	lle	GI n	Met 480
Thr GIn Ser F	Pro Ser 485	Ser Le	eu Ser	Al a	Ser 490	Val	GI y	Asp	Arg	Val 495	Thr
lle Thr Cys S	Ser Ala 500	Ser GI	n Asp	Пе 505	Ser	Asn	Tyr	Leu	Asn 510	Trp	Tyr

Page 57

		ec	olf-seql.txt	
GIn GIn Lys 515	Pro Gly Lys	Ala Pro Lys 520	Val Leu IIe	Tyr Phe Thr Ser 525
Ser Leu His 530	Ser Gly Val	Pro Ser Arg 535	Phe Ser Gly 540	Ser Gly Ser Gly
Thr Asp Phe 545	Thr Leu Thr 550		Leu GIn Pro 555	Glu Asp Phe Ala 560
Thr Tyr Tyr	Cys GIn GIr 565	Tyr Ser Thr	Val Pro Trp 570	Thr Phe Gly Gln 575
Gly Thr Lys	Val Glu Ile 580	E Lys Ser Ser 585	Ala Ser Thr	Lys Gly Pro Ser 590
Val Phe Pro 595	Leu Ala Pro	Ser Ser Lys 600	Ser Thr Ser	Gly Gly Thr Ala 605
Ala Leu Gly 610	Cys Leu Val	Lys Asp Tyr 615	Phe Pro Glu 620	Pro Val Thr Val
Ser Trp Asn 625	Ser GLy ALa 630		Gly Val His 635	Thr Phe Pro Ala 640
Val Leu Gln	Ser Ser Gly 645	' Leu Tyr Ser	Leu Ser Ser 650	Val Val Thr Val 655
Pro Ser Ser	Ser Leu Gly 660	Thr GIn Thr 665	5 5	Asn Val Asn His 670
Lys Pro Ser 675	Asn Thr Lys	Val Asp Lys 680	Lys Val Glu	Pro Lys Ser Cys 685
<210> 40 <211> 450 <212> PRT <213> Arti	ficial Seque	ence		
<veg heav</veg 	sFab-Fab hea F> with VL-V y chain (HC) ine-serine-1	'H domain exc <ang-2> wi</ang-2>) including (hange wild ty ld type (wt)	one heavy chain (HC) ype (wt) coupled to one via
<400> 40				
Asp lle Gln 1	Met Thr Glr 5	Ser Pro Ser	Ser Leu Ser 10	Ala Ser Val Gly 15
Asp Arg Val	Thr lle Thr 20	Cys Ser Ala 25	Ser Gln Asp	lle Ser Asn Tyr 30
Leu Asn Trp 35	Tyr Gln Glr	Lys Pro Gly 40	Lys Ala Pro Page 58	Lys Val Leu IIe 45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly 50 55 60 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr IIe Ser Ser Leu Gln Pro 65 70 75 80 80 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp 85 90 95 Thr Phe Gly Gln Gly Thr Lys Val Glu IIe Lys Ser Ser Ala Ser Thr 100 105 110 Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser 115 120 125 Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu 130 135 140 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His 145 150 155 160 145 160 Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser 165 170 175 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr IIe Cys 180 185 190 Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu 195 200 205 195 Pro Lys Ser Cys Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Glu 210 215 220 Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys 225 230 235 240 Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr Tyr Met His Trp Val Arg 245 250 250 255 GIN Ala Pro Gly GIN Gly Leu Glu Trp Met Gly Trp IIe Asn Pro Asn 260 265 270 Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met 275 280 285 Thr Arg Asp Thr Ser IIe Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu 290 295 300 Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Pro Asn Pro 30Š 310 320 315 Page 59

eolf-seql.txt

	Tyr	Asp	Ser 325	Ser	GI y	Tyr	Tyr	Tyr 330	Pro	GI y	Al a	Phe	Asp 335	lle
Trp Gly	/GIn	GI y 340	Thr	Met	Val	Thr	Val 345	Ser	Ser	Al a	Ser	Thr 350	Lys	GI y
Pro Sei	- Val 355	Phe	Pro	Leu	Al a	Pro 360	Ser	Ser	Lys	Ser	Thr 365	Ser	GI y	GI y
Thr Ala 370		Leu	GI y	Cys	Leu 375	Val	Lys	Asp	Tyr	Phe 380	Pro	GI u	Pro	Val
Thr Val 385	Ser	Тгр	Asn	Ser 390	GI y	Al a	Leu	Thr	Ser 395	GI y	Val	Hi s	Thr	Phe 400
Pro Ala	a Val	Leu	GI n 405	Ser	Ser	GI y	Leu	Tyr 410	Ser	Leu	Ser	Ser	Val 415	Val
Thr Val	Pro	Ser 420	Ser	Ser	Leu	GI y	Thr 425	GI n	Thr	Tyr	lle	Cys 430	Asn	Val
Asn His	5 Lys 435	Pro	Ser	Asn	Thr	Lys 440	Val	Asp	Lys	Lys	Val 445	GI u	Pro	Lys
Ser Cys 450														
	41													
<210> <211> <212> <213>	450 PRT Arti	fi ci a	al Se	equer	nce									
<211> <212>	450 PRT Arti Cross <veg< td=""><td>sFab F> wi y cha</td><td>-Fab th N ain (</td><td>heav /L-VH (HC)</td><td>/y cł ł dor <ang< td=""><td>nain j-2></td><td>exch</td><td>nange</td><td>e wil</td><td>dty</td><td>pe (</td><td>(wt)</td><td>coup</td><td>ain (HC) bled to one tutions via</td></ang<></td></veg<>	sFab F> wi y cha	-Fab th N ain (heav /L-VH (HC)	/y cł ł dor <ang< td=""><td>nain j-2></td><td>exch</td><td>nange</td><td>e wil</td><td>dty</td><td>pe (</td><td>(wt)</td><td>coup</td><td>ain (HC) bled to one tutions via</td></ang<>	nain j-2>	exch	nange	e wil	dty	pe ((wt)	coup	ain (HC) bled to one tutions via
<211> <212> <213> <220>	450 PRT Arti Cross <veg heav</veg 	sFab F> wi y cha	-Fab th N ain (heav /L-VH (HC)	/y cł ł dor <ang< td=""><td>nain j-2></td><td>exch</td><td>nange</td><td>e wil</td><td>dty</td><td>pe (</td><td>(wt)</td><td>coup</td><td>oled to one</td></ang<>	nain j-2>	exch	nange	e wil	dty	pe ((wt)	coup	oled to one
<211> <212> <213> <220> <223>	450 PRT Arti <veg heav glyc 41</veg 	sFab F> wi y cha i ne-s	-Fab th \ ain (serir	heav /L-Vł (HC) ne-li	/y cł ł dor <ang nker</ang 	nain g-2> ⁻s	exch with	nange n K14	e wil 47E a	d ty and H	/pe((213]	(wt) E suk	coup osti 1	oled to one tutions via
<211> <212> <213> <220> <223> <400>	450 PRT Arti Cross <vegi heav gl yc 41 e GI n</vegi 	sFab F> wi y cha ne-s Met	-Fab th N ain (serir Thr 5	heav /L-Vł (HC) ne-li	/y ch don <ang nker Ser</ang 	nain j-2> ^s Pro	exch with Ser	ser	e wil 47E a Leu	d ty and F Ser	ype ((213) Al a	(wt) sub	coup osti 1 Val 15	oled to one tutions via Gly
<211> <212> <213> <220> <220> <223> <400> Asp II(450 PRT Arti <vegi heavy glyc 41 e GIn</vegi 	sFab F> wi y cha i ne-s Met Thr 20	-Fab th \ ain (serir 5 Ile	heav /L-Vł (HC) ne-li Gln Thr	/y cł ł don <ang nker Ser Cys</ang 	nain g-2> °s Pro Ser	exch with Ser Al a 25	Ser 10 Ser 5	e wil 17E a Leu GIn	d ty and F Ser Asp	ype ((2138 Ala Ile	(wt) sub Ser Ser 30	Cour Disti 15 Asn	oled to one tutions via Gly Tyr
<211> <212> <213> <220> <223> <400> Asp II (1 Asp Arg	450 PRT Arti VEG heav glyc 41 e GIn y Val	sFab F> wi y cha i ne-s Met Thr 20 Tyr	-Fab th N ain (serin 5 Ile GIn	heav /L-VF (HC) ne-li GIn Thr	/y ch don <ang nker Ser Cys Lys</ang 	nain g-2> s Pro Ser Pro 40	exch with Ser Al a 25 Gl y	Ser 10 Ser Lys	e wil 17E a Leu GIn AIa	d ty and F Ser Asp Pro	Ala Ile Lys	(wt) sut Ser 30 Val	Val 15 Asn Leu	oled to one tutions via Gly Tyr Ile

65	70	eol f-seql . txt 75	80	
	Thr Tyr Tyr C <u>y</u> 35	ys Gln Gln Tyr Ser 90	Thr Val Pro Tr 95	р
Thr Phe Gly Gln G 100	Gly Thr Lys Va	al Glu IIe Lys Ser 105	Ser Ala Ser Th 110	r
Lys Gly Pro Ser V 115		eu Ala Pro Ser Ser 20	Lys Ser Thr Se 125	r
Gly Gly Thr Ala A 130	Ala Leu Gly Cy 135	ys Leu Val Lys Asp 140	Tyr Phe Pro Gl	u
Pro Val Thr Val S 145	Ser Trp Asn Se 150	er Gly Ala Leu Thr 155	Ser Gly Val Hi 16	
	/al Leu Gln Se 65	er Ser Gly Leu Tyr 170	Ser Leu Ser Se 175	r
Val Val Thr Val P 180	Pro Ser Ser Se	er Leu Gly Thr Gln 185	Thr Tyr IIe Cy 190	S
Asn Val Asn His L 195		sn Thr Lys Val Asp 00	Lys Lys Val Gl 205	u
Pro Lys Ser Cys S 210	Ger Gly Gly Gl 215	ly Gly Ser Gln Val 220	Gln Leu Val Gl	u
Ser Gly Ala Glu V 225	/al Lys Lys Pi 230	ro Gly Ala Ser Val 235	Lys Val Ser Cy 24	
	yr Thr Phe Tl 245	hr Gly Tyr Tyr Met 250	His Trp Val Ary 255	g
GIn Ala Pro GIy G 260	GIN GIY Leu GI	lu Trp Met Gly Trp 265	lle Asn Pro As 270	n
Ser Gly Gly Thr A 275		In Lys Phe GIn GIy 80	Arg Val Thr Me 285	t
Thr Arg Asp Thr S 290	Ser IIe Ser TH 295	hr Ala Tyr Met Glu 300	Leu Ser Arg Le	u
Arg Ser Asp Asp T 305	hr Ala Val Ty 310	yr Tyr Cys Ala Arg 315	Ser Pro Asn Pro 32	
	Ser Ser Gly Ty 325	yr Tyr Tyr Pro Gly 330	Ala Phe Asp II 335	е
Trp Gly Gln Gly T	hr Met Val Th	hr Val Ser Ser Ala Page 61	Ser Thr Lys Gl	у

		340					eo 345	lf-s	eqI.	txt		350		
Pro Se	r Val 355	Phe	Pro	Leu	Al a	Pro 360	Ser	Ser	Lys	Ser	Thr 365	Ser	GI y	GI y
Thr Al a 370		Leu	GI y	Cys	Leu 375	Val	GI u	Asp	Tyr	Phe 380	Pro	GI u	Pro	Val
Thr Va 385	Ser	Тгр	Asn	Ser 390	GI y	Al a	Leu	Thr	Ser 395	GI y	Val	Hi s	Thr	Phe 400
Pro Ala	a Val	Leu	GI n 405	Ser	Ser	GI y	Leu	Tyr 410	Ser	Leu	Ser	Ser	Val 415	Val
Thr Va	Pro	Ser 420	Ser	Ser	Leu	GI y	Thr 425	GI n	Thr	Tyr	lle	Cys 430	Asn	Val
Asn His	s Lys 435	Pro	Ser	Asn	Thr	Lys 440	Val	Asp	GI u	Lys	Val 445	GI u	Pro	Lys
Ser Cy: 450														
<210> <211> <212>	42 667 PRT													
<212> <213>	Arti	ficia	al Se	equer	nce									
	Arti Cross	sFabi F> wi y cha	2-Fak th N ain () hea /L-Vł (HC)	avy o I dor ≺Ang	nain g-2>	exch	nange	e wil	d ty	ype (nains (HC) oled to one
<213> <220>	Arti Cross <veg heavy</veg 	sFabi F> wi y cha	2-Fak th N ain () hea /L-Vł (HC)	avy o I dor ≺Ang	nain g-2>	exch	nange	e wil	d ty	ype (
<213> <220> <223>	Arti Cross <veg heav gl yc 42</veg 	sFab2 F> wi y cha i ne-s	2-Fat th \ ain (serir	o hea /L-Vł (HC) nee-I	avy o I don ≺Ano inke	nain g-2> ers	excł wi l	ange d ty	e wil ype	d ty (wt)	ype vi a	(wt)	coup	oled to one
<213> <220> <223> <400>	Arti Cros: <vegi heav glyc 42 e GIn</vegi 	sFab2 F> wi y cha i ne-s Met	2-Fab th \ ain (serir Thr 5	o hea /L-Vł (HC) nee-I GI n	avy o I don <ano i nko Ser</ano 	nain g-2> ers Pro	exch will Ser	iange d ty Ser 10	e wil ype Leu	d ty (wt) Ser	ype via Ala	(wt) Ser	čour Val 15	oled to one Gly
<213> <220> <223> <400> Asp II (1	Arti Cros: <veg heav glyc 42 e Gln g Val</veg 	sFab2 F> wi y cha i ne-s Met Thr 20	2-Fat th \ ain o serir 5 Ile	o hea /L-VH (HC) nee-I GI n Thr	avy o l don <ang i nke Ser Cys</ang 	nain g-2> ers Pro Ser	exch wil Ser Al a 25	Ser 10 Ser	e wil ype Leu GIn	d ty (wt) Ser Asp	ype via Ala Ile	(wt) Ser Ser 30	čour Val 15 Asn	Gly Tyr
<213> <220> <223> <400> Asp II 1 Asp Arg	Arti Cros: <veg heav glyc 42 e Gln g Val n Trp 35</veg 	sFab2 F> wi y cha i ne-s Met Thr 20 Tyr	2-Fat th N ain o serir Thr 5 IIe GIn	GI n GI n GI n	Avy of don <ang i nke Ser Cys Lys</ang 	nain g-2> ers Pro Ser Pro 40	exch wil Ser Al a 25 Gl y	Ser 10 Ser Lys	e wil ype GIn AIa	d ty (wt) Ser Asp Pro	Ala Ile Lys 45	(wt) Ser 30 Val	Cour Val 15 Asn Leu	Gly Tyr Ile
<213> <220> <223> <400> Asp II 1 Asp Arg Leu Asi Tyr Pho	Arti Cross <veg heavy glyc 42 e GIn g Val n Trp 35 e Thr</veg 	sFab2 F> wi y cha i ne-s Met Thr 20 Tyr Ser	2-Fat th N ain o serir Thr 5 IIe GIn Ser	Gl n Gl n Gl n Leu	Avy of dom <ang i nke Ser Cys Lys Hi s 55</ang 	nain g-2> ers Pro Ser Pro 40 Ser	exch wil Ser Al a 25 Gl y Gl y	Ser 10 Ser Lys Val	e wil ype GIn Ala Pro	d ty (wt) Ser Asp Pro Ser 60	via Ala Ile Lys 45 Arg	(wt) Ser Ser 30 Val Phe	Cour Val 15 Asn Leu Ser	Gly Tyr Ile Gly

eolf-seql.txt Thr Phe Gly Gln Gly Thr Lys Val Glu IIe Lys Ser Ser Ala Ser Thr 100 105 110 Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser 115 120 125 115 Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu 130 135 140 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His 145 150 155 160 145 160 Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser 165 170 175 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr IIe Cys 180 185 190 Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu 195 200 205 200 195 Pro Lys Ser Cys Ser Gly Gly Gly Gly Ser Asp IIe Gln Met Thr Gln 210 215 220 Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr 225 230 235 240 Cys Ser Ala Ser Gin Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gin Gin 245 250 255 Lys Pro Gly Lys Ala Pro Lys Val Leu IIe Tyr Phe Thr Ser Ser Leu 260 265 270 265 His Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp 275 280 285 Phe Thr Leu Thr IIe Ser Ser Leu GIn Pro GIu Asp Phe Ala Thr Tyr 290 295 300 290 Tyr Cys GIn GIn Tyr Ser Thr Val Pro Trp Thr Phe GIy GIn GIy Thr 305 310 315 320 Lys Val Glu IIe Lys Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe 325 330 335 Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu 340 345 350 Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp 355 360 365

eolf-seql.txt Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu 370 375 380 GIn Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser 385 390 395 400 Ser Ser Leu Gly Thr Gln Thr Tyr IIe Cys Asn Val Asn His Lys Pro 405 410 415 Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Gly Gly 420 425 430 Gly Gly Ser Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys 435 440 445 Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe 450 455 460 Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu 470 465 475 480 Glu Trp Met Gly Trp IIe Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala 485 490 495 GIn Lys Phe GIn GIy Arg Val Thr Met Thr Arg Asp Thr Ser IIe Ser 500 Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val 515 520 525 Tyr Tyr Cys Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly 530 535 540 Tyr Tyr Tyr Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val 545 550 555 560 560 Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala 565 570 575 Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu 580 590 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly 595 600 605 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser 610 615 620 Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Leu 625 630 635 640

eolf-seql.txt Gly Thr Gln Thr Tyr IIe Cys Asn Val Asn His Lys Pro Ser Asn Thr 645 650 655 650 655 Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys 660 665 <210> 43 <211> 667 <212> PRT Artificial Sequence <213> <220> <223> CrossFab2-Fab heavy chain (HC) including two heavy chains (HC) <VEGF> with VL-VH domain exchange wild type (wt) coupled to one heavy chain (HC) <Ang-2> with K147E and K231E substitutions via gl yci ne-seri ne-l i nkers <400> 43 Asp Ile GIn Met Thr GIn Ser Pro Ser Ser Leu Ser Ala Ser Val Gly 5 10 Asp Arg Val Thr IIe Thr Cys Ser Ala Ser Gln Asp IIe Ser Asn Tyr 20 25 30 20 Leu Asn Trp Tyr GIn GIn Lys Pro GIy Lys Ala Pro Lys Val Leu IIe 35 40 45 Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly 50 55 60 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr IIe Ser Ser Leu Gln Pro 65 70 75 80 65 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp 85 90 95 Thr Phe Gly Gln Gly Thr Lys Val Glu IIe Lys Ser Ser Ala Ser Thr 100 105 110 Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser 125 115 120 Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu 130 135 140 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val 145 150 155 His 160 Thr Phe Pro Al a Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser165170175 165 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr IIe Cys 180 185 190

			eol f-s	eql.txt			
Asn Val Asn H 195	lis Lys Pro	Ser Asn ⁻ 200	Thr Lys	Val Asp	Lys Lys 205	Val G	il u
Pro Lys Ser C 210	Cys Ser Gly	GlyGly(215	Gly Ser	Asp IIe 220	Gln Met	Thr G	il n
Ser Pro Ser S 225	Ser Leu Ser 230	Ala Ser V	Val Gly	Asp Arg 235	Val Thr		⁻hr 240
Cys Ser Ala S	Ser GLn Asp 245	lle Ser /	Asn Tyr 250	Leu Asn	Trp Tyr	GIn G 255	3l n
Lys Pro Gly L 2	_ys Ala Pro 260		Leu IIe 265	Tyr Phe	Thr Ser 270	Ser L	eu
His Ser Gly V 275	/al Pro Ser	Arg Phe S 280	Ser Gly	Ser Gly	Ser Gly 285	Thr A	lsp
Phe Thr Leu T 290	Thr Ile Ser	Ser Leu (295	GIn Pro	Glu Asp 300	Phe Ala	Thr T	yr
Tyr Cys Gln G 305	GIn Tyr Ser 310	Thr Val I	Pro Trp	Thr Phe 315	Gly Gln		⁻hr 820
Lys Val Glu I	le Lys Ser 325	Ser Ala S	Ser Thr 330	Lys Gly	Pro Ser	Val P 335	he
Pro Leu Ala F 3	Pro Ser Ser 340		Thr Ser 345	Gly Gly	Thr Ala 350	Ala L	eu
Gly Cys Leu V 355	/al Lys Asp	Tyr Phe I 360	Pro Glu	Pro Val	Thr Val 365	Ser T	rp
Asn Ser Gly A 370	Ala Leu Thr	Ser Gly V 375	Val His	Thr Phe 380	Pro Ala	Val L	eu
GIn Ser Ser G 385	Gly Leu Tyr 390	Ser Leu S	Ser Ser	Val Val 395	Thr Val		Ser 100
Ser Ser Leu G	Gly Thr Gln 405	Thr Tyr	lle Cys 410	Asn Val	Asn His	Lys P 415	ro
Ser Asn Thr L 4	_ys Val Asp 120		Val Glu 425	Pro Lys	Ser Cys 430	GIyG	91 y
GlyGlySerC 435	GIn Val GIn	Leu Val (440	Glu Ser	Gly Ala	GLu Val 445	Lys L	ys
Pro Gly Ala S 450	Ser Val Lys	Val Ser (455	Cys Lys	Ala Ser 460	Gly Tyr	Thr P	he

			eol f-s	seql.txt		
Thr Gly Tyr Ty 465	^ Met His 470	Trp Val	Arg GIn	Ala Pro 475	Gly Gln	GLY Leu 480
Glu Trp Met Gl	y Trp Ile 485	Asn Pro	Asn Ser 490	GIy GIy	Thr Asn	Tyr Ala 495
GIn Lys Phe GI 50		Val Thr	Met Thr 505	Arg Asp	Thr Ser 510	
Thr Ala Tyr Me 515	t Glu Leu	Ser Arg 520		Ser Asp	Asp Thr 525	Ala Val
Tyr Tyr Cys Al 530	a Arg Ser	Pro Asn 535	Pro Tyr	Tyr Tyr 540	Asp Ser	Ser Gly
Tyr Tyr Tyr Pr 545	o GLy ALa 550	Phe Asp	lle Trp	GIy GIn 555	Gly Thr	Met Val 560
Thr Val Ser Se	r Ala Ser 565	Thr Lys	GI y Pro 570	Ser Val	Phe Pro	Leu Ala 575
Pro Ser Ser Ly 58		Ser Gly	Gly Thr 585	Ala Ala	Leu GIy 590	
Val Glu Asp Ty 595	r Phe Pro	Glu Pro 600		Val Ser	Trp Asn 605	Ser Gly
Ala Leu Thr Se 610	^ Gly Val	His Thr 615	Phe Pro	Ala Val 620		Ser Ser
Gly Leu Tyr Se 625	- Leu Ser 630	Ser Val	Val Thr	Val Pro 635	Ser Ser	Ser Leu 640
Gly Thr Gln Th	r Tyr lle 645	Cys Asn	Val Asn 650	His Lys	Pro Ser	Asn Thr 655
Lys Val Asp Gl 66		Glu Pro	Lys Ser 665	Cys		
<210> 44 <211> 439 <212> PRT <213> Artific	al Sequei	nce				
<220> <223> heavy c substit	nain (HC) ution	<vegf></vegf>	with VH-	VL excha	nge with	K147E
<400> 44						
Asp lle Gln Me 1	t Thr Gln 5	Ser Pro	Ser Ser 10	Leu Ser	Ala Ser	Val Gly 15

eolf-seql.txt Asp Arg Val Thr IIe Thr Cys Ser Ala Ser Gin Asp IIe Ser Asn Tyr 20 25 30 Leu Asn Trp Tyr GIn GIn Lys Pro GIy Lys Ala Pro Lys Val Leu IIe 35 40 45 Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly 50 55 60 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr IIe Ser Ser Leu Gln Pro 65 70 75 80 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp 85 90 95 Thr Phe Gly Gln Gly Thr Lys Val Glu IIe Lys Ser Ser Ala Ser Thr 100 105 110 100 Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser 115 120 125 Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Glu Asp Tyr Phe Pro Glu 130 135 140 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His 145 150 155 160 160 Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser 165 170 175 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr IIe Cys 180 185 190 Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu 195 200 205 Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro 210 215 220 Glu Leu Cly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys 225 230 235 240 Asp Thr Leu Met IIe Ser Arg Thr Pro Glu Val Thr Cys Val Val Val 245 250 255 Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp 260 265 270 Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr 275 280 285

eolf-seql.txt Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His GIn Asp 290 295 300
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu 305 310 315 320
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg 325 330 335
Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys 340 345 350
Asn GIn Val Ser Leu Ser Cys Ala Val Lys GIy Phe Tyr Pro Ser Asp 355 360 365
IIe Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys 370 375 380
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser 385 390 395 400
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser 405 410 415
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser 420 425 430
Leu Ser Leu Ser Pro Gly Lys 435
<210> 45 <211> 230 <212> PRT <213> Artificial Sequence
<220> <223> light chain (LC) <vegf> with VH-VL exchange with Q124K substitution</vegf>
<400> 45
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly 1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr 20 25 30
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35 40 45
Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe 50 55 60

65	70	olf-seqL.txt 75	80
Leu GIn Met Asn Ser	Leu Arg Ala Glu	Asp Thr Ala Val Tyr	Tyr Cys
85		90	95
Ala Lys Tyr Pro His	Tyr Tyr Gly Ser	Ser His Trp Tyr Phe	Asp Val
100	105	110	
Trp Gly Gln Gly Thr	Leu Val Thr Val	Ser Ser Ala Ser Val	Ala Ala
115	120	125	
Pro Ser Val Phe IIe	Phe Pro Pro Ser	Asp GLu Lys Leu Lys	Ser Gly
130	135	140	
Thr Ala Ser Val Val	Cys Leu Leu Asn	Asn Phe Tyr Pro Arg	Glu Ala
145	150	155	160
Lys Val GIn Trp Lys		Leu GIn Ser Gly Asn	Ser GIn
165		170	175
Glu Ser Val Thr Glu	Gln Asp Ser Lys	Asp Ser Thr Tyr Ser	Leu Ser
180	185	190	
Ser Thr Leu Thr Leu	Ser Lys Ala Asp	Tyr Glu Lys His Lys	Val Tyr
195	200	205	
Ala Cys Glu Val Thr	His GIn Gly Leu	Ser Ser Pro Val Thr	Lys Ser
210	215	220	
Phe Asn Arg Gly Glu 225	Cys 230		
<210> 46 <211> 439 <212> PRT <213> Artificial S	equence		
<220> <223> heavy chain substitution		VH-VL exchange with	K147E, and K213E
<400> 46			
Asp lle Gln Met Thr	Gln Ser Pro Ser	Ser Leu Ser Ala Ser	Val GLy
1 5		10	15
Asp Arg Val Thr Ile	Thr Cys Ser Ala	Ser Gln Asp Ile Ser	Asn Tyr
20	25	30	
Leu Asn Trp Tyr Gln	GIn Lys Pro GIy	Lys Ala Pro Lys Val	Leu IIe
35	40	45	
Tyr Phe Thr Ser Ser 50	Leu His Ser Gly 55	Val Pro Ser Arg Phe 60 Page 70	Ser Gly

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr IIe Ser Ser Leu Gln Pro 65 70 75 80 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu IIe Lys Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser 115 120 125 Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Glu Asp Tyr Phe Pro Glu 130 135 140 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 165 170 175 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr IIe Cys 180 185 190 Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Glu 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 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met IIe Ser Arg Thr Pro Glu Val Thr Cys Val Val Val 245 250 255 Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp 260 265 270 Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr 275 280 285 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 305 310 315 320 Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Page 71

Glu Pro Gln	Val Cys 340	Thr L	Leu Pro	Pro 345	Ser	Arg	Asp	GI u	Leu 350	Thr	Lys
Asn GIn Val 355	Ser Leu	Ser (Cys Ala 360		Lys	GI y	Phe	Tyr 365	Pro	Ser	Asp
lle Ala Val 370	Glu Trp		Ser Asn 375	GI y	Gl n	Pro	GI u 380	Asn	Asn	Tyr	Lys
Thr Thr Pro 385	Pro Val	Leu <i>4</i> 390	Asp Ser	Asp	GI y	Ser 395	Phe	Phe	Leu	Val	Ser 400
Lys Leu Thr	Val Asp 405	Lys S	Ser Arg	Тгр	Gl n 410	Gl n	GI y	Asn	Val	Phe 415	Ser
	Met His 420	Glu A	Ala Leu	Hi s 425	Asn	Hi s	Tyr	Thr	GI n 430	Lys	Ser
Leu Ser Leu 435	Ser Pro	GIyl	Lys								
<210> 47 <211> 230 <212> PRT <213> Artif	icial S	equenc	се								
	chain itution	(LC) <	<vegf></vegf>	wi th	VH-V	/L e>	char	nge v	vi th	E123	3K, and Q124K
<223> light		(LC) <	<vegf></vegf>	wi th	VH-V	/Le>	char	nge v	vi th	E123	3K, and Q124K
<223> light subst	i tuti on										
<223> light subst <400> 47 Glu Val Gln 1 Ser Leu Arg	i tuti on Leu Val 5	Glus	Ser Gly	GI y	GI y 10	Leu	Val	GIn	Pro	GI y 15	GI y
<223> light subst <400> 47 Glu Val Gln 1 Ser Leu Arg	itution Leu Val 5 Leu Ser 20	GI u S Cys <i>F</i>	Ser Gly Ala Ala	GI y Ser 25	GI y 10 GI y	Leu Tyr	Val Thr	GI n Phe	Pro Thr 30	GI y 15 Asn	GI y Tyr
<223> light subst <400> 47 Glu Val Gln 1 Ser Leu Arg Gly Met Asn	i tuti on Leu Val 5 Leu Ser 20 Trp Val	Glu S Cys A Arg C Tyr 1	Ser Gly Ala Ala Gln Ala 40	GI y Ser 25 Pro	GI y 10 GI y GI y	Leu Tyr Lys	Val Thr Gl y	GI n Phe Leu 45	Pro Thr 30 GI u	GI y 15 Asn Trp	GI y Tyr Val
<223> light subst <400> 47 Glu Val Gln 1 Ser Leu Arg Gly Met Asn 35 Gly Trp Ile	itution Leu Val 5 Leu Ser 20 Trp Val Asn Thr	Glu S Cys A Arg C Tyr 1	Ser Gly Ala Ala Gln Ala 40 Thr Gly 55	GI y Ser 25 Pro GI u	GI y GI y GI y Pro	Leu Tyr Lys Thr	Val Thr Gl y Tyr 60	GI n Phe Leu 45 AI a	Pro Thr 30 GI u AI a	GI y 15 Asn Trp Asp	GI y Tyr Val Phe
<223> light subst <400> 47 Glu Val Gln 1 Ser Leu Arg Gly Met Asn 35 Gly Trp Ile 50 Lys Arg Arg	itution Leu Val Leu Ser 20 Trp Val Asn Thr Phe Thr	Glu S Cys A Arg C Tyr 1 Phe S 70	Ser Gly Ala Ala Gln Ala 40 Thr Gly 55 Ser Leu	GI y Ser 25 Pro GI u Asp	GI y GI y GI y Pro Thr	Leu Tyr Lys Thr Ser 75	Val Thr Gly Tyr 60 Lys	GI n Phe Leu 45 AI a Ser	Pro Thr 30 GI u AI a Thr	GI y 15 Asn Trp Asp AI a	GI y Tyr Val Phe Tyr

	eol f	f-seql.txt	
Trp Gly Gln Gly Thr	Leu Val Thr Val So	Ger Ser Ala Ser Val A	Ala Ala
115	120	125	
Pro Ser Val Phe IIe	Phe Pro Pro Ser A	sp Lys Lys Leu Lys S	Ser Gly
130	135	140	
Thr Ala Ser Val Val	Cys Leu Leu Asn A	asn Phe Tyr Pro Arg G	Glu Ala
145	150	155	160
Lys Val Gln Trp Lys		eu GIn Ser GIy Asn S	Ser Gln
165		70 1	175
Glu Ser Val Thr Glu	GIn Asp Ser Lys A	asp Ser Thr Tyr Ser L	_eu Ser
180	185	190	
Ser Thr Leu Thr Leu	Ser Lys Ala Asp Ty	yr Glu Lys His Lys V	/al Tyr
195	200	205	
Ala Cys Glu Val Thr	His GIn Gly Leu So	Ger Ser Pro Val Thr L	.ys Ser
210	215	220	
Phe Asn Arg Gly Glu 225	Cys 230		