

## ANTIBODY THAT BINDS TO VEGF AND IL-1BETA AND METHODS OF USE

### FIELD OF THE INVENTION

5       The present invention relates to anti-VEGF/anti-IL-1beta antibodies and methods of using the same.

### BACKGROUND OF THE INVENTION

10       A bispecific antibody binding to IL-1beta and VEGF has been reported previously and was suggested for treatment of ocular vascular diseases (WO2016/075034, antibody "0032"). The bispecific anti-VEGF/anti-IL-1beta antibody 0032 is a full length IgG-like antibody with a VH/VL domain exchange in one binding arm (WO2009/080252, Schaefer, W. et al, PNAS, 108 (2011) 11187-1191), wherein the binding arm of the wild type antibody domain arrangement  
15       specifically binds to IL-1beta and the binding arm comprising the VH/VL domain crossover specifically binds to VEGF. The VEGF binding arm comprises the VH and VL domains of anti-VEGF antibody ranibizumab.

20       Multispecific antibodies comprising two paratopes in one pair of a variable heavy chain domain (VH) and a variable light chain domain (VL) have been described in WO2008/027236; WO2010/108127 and Bostrom, J., et al., Science 323 (2009) 1610-1614 as well as in WO2012/163520.

25       WO2012/163520 discloses bispecific antibodies comprising two non-overlapping paratopes in one pair of VH and VL domains ("DutaFabs"). Each paratope of the bispecific antibody of WO2012/163520 comprises amino acids from the heavy chain and from the light chain CDRs, wherein heavy chain CDR-H1 and CDR-H3 as well as light chain CDR-L2 contribute to the first paratope and light chain CDR-L1 and CDR-L3 as well as heavy chain CDR-H2 contribute to the second paratope. Monospecific antibodies comprising the individual paratopes are isolated independently from different Fab-libraries, which are diversified in either  
30       the first or the second paratope. The amino acid sequences of said monospecific antibodies are identified and merged into the biparatopic VH and VL pair. One

exemplary Fab fragment specifically binding to VEGF and IL-6 is disclosed in WO2012/163520.

There is a need for improved therapeutic antibodies that bind to VEGF and IL-1beta.

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## SUMMARY OF THE INVENTION

The present invention relates to bispecific anti-VEGF/anti-IL-1beta antibodies and methods of using the same.

10 In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, comprising a VEGF paratope and an IL-1beta paratope within one cognate pair of a variable light chain domain (VL domain) and a variable heavy chain domain (VH domain), wherein the VEGF paratope comprises amino acid residues from CDR-H2, CDR-L1 and CDR-L3 of the antibody, wherein the IL-1beta paratope comprises amino acid residues from the CDR-H1, CDR-H3 and  
15 CDR-L2 of the antibody.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, comprising a VEGF paratope and an IL-1beta paratope within one cognate pair of a VL domain and a VH domain, wherein the pair of the variable light chain domain and the variable heavy chain domain simultaneously  
20 binds to human VEGF and human IL-1beta.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, comprising a VEGF paratope and an IL-1beta paratope within one cognate pair of a VL domain and a VH domain, wherein none of the amino acids that are comprised in the VEGF paratope are comprised in the IL-1beta  
25 paratope.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, comprising a VEGF paratope and an IL-1beta paratope within one cognate pair of a VL domain and a VH domain, wherein the antibody binds to the same epitope on human VEGF and to the same epitope on human IL-1beta as an antibody with a variable heavy chain domain of SEQ ID NO: 11 and a  
30 variable light chain domain of SEQ ID NO: 12.

5 In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein an antibody Fab fragment of the antibody binds (i) to human VEGF121 with a  $K_D$  of less than 10 pM as measured by surface plasmon resonance, and (ii) to human IL-1beta with a  $K_D$  of less than 30 pM as measured by surface plasmon resonance.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein an antibody Fab fragment of the antibody exhibits an aggregation onset temperature of more than 70 °C.

10 In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein an antibody Fab fragment of the antibody exhibits a melting temperature of more than 80 °C as measured by dynamic light scattering.

15 In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein binding of an antibody Fab fragment of the antibody to human VEGF inhibits binding of VEGF to VEGFR2 with an  $IC_{50}$  of less than 50 nM as measured by surface plasmon resonance; and wherein binding of an antibody Fab fragment of the antibody to human IL-1beta inhibits binding of IL-1beta to IL-1betaR1 with an  $IC_{50}$  of less than 30 nM as measured by surface plasmon resonance.

20 In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8.

25 In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid

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sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.

5           In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, comprising a VH domain comprising amino acid residues E2, G26, V28, K30, W31, N35b, D35c, K52a, D55, H56, Y58, T61, K62, F63, I64, R66, R83, K94, D95, V96, F98, and D101, and a VL domain comprising amino acid residues I2, Y27, W27a, S27c, S27d, L32, Y49, D50, Y53, K54, L56, G57, E67,  
10           D68, Q69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the VH and VL domains is according to the Kabat numbering system. In one embodiment the antibody comprises a VEGF paratope comprising the following amino acid residues in the VH domain D55, H56, Y58, T61, K62, F63, I64, R66, and R83, and the following amino acid residues in the VL domain I2, Y27, W27a, S27c, S27d, E67,  
15           D68, Q69, R92, Y93, H94, and Y96; and an IL-1beta paratope comprising the following amino acid residues in the VH domain E2, G26, V28, K30, W31, N35b, D35c, K52a, K94, D95, V96, F98, and D101, and the following amino acid residues in the VL domain L32, Y49, D50, Y53, K54, L56, G57, Y91.

          In one aspect the invention provides an antibody that binds to human VEGF  
20           and to human IL-1beta, comprising a VH domain comprising amino acid residues E2, G26, V28, K30, W31, N35b, D35c, K52a, D55, H56, Y58, T61, K62, F63, I64, R66, R83, K94, D95, V96, F98, and D101, and a VL domain comprising amino acid residues I2, Y27, W27a, S27c, S27d, L32, Y49, D50, Y53, K54, L56, G57, S67, H68, E69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the VH and VL  
25           domains is according to the Kabat numbering system. In one embodiment the antibody comprises a VEGF paratope comprising the following amino acid residues in the VH domain D55, H56, Y58, T61, K62, F63, I64, R66, and R83, and the following amino acid residues in the VL domain I2, Y27, W27a, S27c, S27d, S67, H68, E69, R92, Y93, H94, and Y96; and an IL-1beta paratope comprising the  
30           following amino acid residues in the VH domain E2, G26, V28, K30, W31, N35b, D35c, K52a, K94, D95, V96, F98, and D101, and the following amino acid residues in the VL domain L32, Y49, D50, Y53, K54, L56, G57, Y91.

          In one aspect the invention provides an antibody that binds to human VEGF  
and to human IL-1beta, comprising (a) a VH domain comprising an amino acid  
35           sequence having at least 90% sequence identity to the amino acid sequence of SEQ

ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12.

5 In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12.

15 In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11, wherein the VH domain comprises amino acid residues E2, G26, V28, K30, R66, R83, and K94; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12, wherein the VL domain comprises amino acid residues I2, Y49, G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.

30 In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1

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comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, comprising (a) a VH domain comprising an amino acid sequence of SEQ ID NO:11 with up to 15 amino acid substitutions; and (b) a variable light chain domain comprising an amino acid sequence of SEQ ID NO:12 with up to 15 amino acid substitutions. In one embodiment the antibody comprises (a) a VH domain comprising an amino acid sequence of SEQ ID NO:11 with up to 15 amino acid substitutions, wherein the amino acid substitutions are located at positions 3 to 25, 36 to 49, 97 to 82c, 84 to 93, or 103 to 113 of SEQ ID NO:11; and (b) a variable light chain domain comprising an amino acid sequence of SEQ ID NO:12 with up to 15 amino acid substitutions, wherein the amino acid substitutions are located at positions 1, 4, 6, 8 to 23, 35 to 48, 58 to 66, 70 to 88, or 98 to 107 of SEQ ID NO:12, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence of SEQ ID NO:11 with up to 15 amino acid substitutions; and (b) a variable light chain domain comprising an amino acid sequence of SEQ ID NO:12 with up to 15 amino acid substitutions.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, and comprising (a) a VH domain comprising an amino acid sequence of SEQ ID NO:11 with up to 15 amino acid substitutions; and (b) a variable light chain domain comprising an amino acid sequence of SEQ ID NO:12 with up to 15 amino acid substitutions.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, comprising a VH sequence of SEQ ID NO:11 and a VL sequence of SEQ ID NO:12.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, comprising a heavy chain amino acid sequence of SEQ ID NO:20 and a light chain amino acid sequence of SEQ ID NO:19.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, comprising a heavy chain amino acid sequence of SEQ ID NO:18 and a light chain amino acid sequence of SEQ ID NO:19.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid

sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, wherein an antibody Fab fragment of the antibody binds (i) to human VEGF121 with a  $K_D$  of less than 10 pM as measured by surface plasmon resonance, and (ii) to human IL-1beta with a  $K_D$  of less than 30 pM as measured by surface plasmon resonance.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12; wherein an antibody Fab fragment of the antibody binds (i) to human VEGF121 with a  $K_D$  of less than 10 pM as measured by surface plasmon resonance, and (ii) to human IL-1beta with a  $K_D$  of less than 30 pM as measured by surface plasmon resonance.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12; wherein an antibody Fab fragment of the antibody exhibits an aggregation onset temperature of more than 70 °C.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a (VL domain) comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12; wherein an antibody Fab fragment of the antibody exhibits a melting temperature of more than 80 °C as measured by dynamic light scattering.

In one aspect the invention provides an antibody that binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12; and wherein binding of an antibody Fab fragment of the antibody to human IL-1beta inhibits binding of IL-1beta to IL-1betaR1 with an IC50 of less than 30 nM as measured by surface plasmon resonance.

One embodiment of the invention relates to an antibody fragment that binds to human VEGF and to human IL-1beta. One embodiment of the invention relates to a bispecific antibody fragment that binds to human VEGF and to human IL-1beta. In one embodiment the antibody fragment is selected from Fv, Fab, Fab', Fab'-SH, F(ab')<sub>2</sub> or single chain antibodies derived therefrom. One embodiment of the invention relates to a Fab fragment that binds to human VEGF and to human IL-1beta. One embodiment of the invention relates to an Fv fragment that binds to human VEGF and to human IL-1beta.

One embodiment of the invention relates to a full length IgG antibody that binds to human VEGF and to human IL-1beta.

In one aspect the invention provides an isolated nucleic acid encoding the antibody of the invention.

5 In one aspect the invention provides a host cell comprising the nucleic acid of the invention.

In one aspect the invention provides an expression vector comprising the nucleic acid of the invention.

10 In one aspect the invention provides a method of producing an antibody that binds to human VEGF and to human IL-1beta comprising culturing the host cell of the invention so that the antibody is produced.

In one aspect the invention provides the antibody produced by the method of the invention.

15 In one aspect the invention provides a pharmaceutical formulation comprising the antibody of the invention and a pharmaceutically acceptable carrier.

In one aspect the invention provides the antibody of the invention for use as a medicament, in one embodiment for use in the treatment of a vascular disease.

20 In one aspect the invention provides the use of the antibody of the invention or the pharmaceutical composition of the invention in the manufacture of a medicament, in one embodiment a medicament for the treatment of a vascular disease.

In one aspect the invention provides a method of treating an individual having a vascular disease comprising administering to the individual an effective amount of the antibody of the invention or the pharmaceutical composition of the invention.

25 In one aspect the invention provides a method of inhibiting angiogenesis in an individual comprising administering to the individual an effective amount of the antibody of the invention or the pharmaceutical composition of the invention to inhibit angiogenesis

30 According to the invention a therapeutic anti-VEGF/anti-IL-1beta antibody is provided that is capable of binding to its target antigens simultaneously, even when

provided as a bispecific Fab fragment. In addition the antibody of the invention provides several valuable properties that allow its therapeutic application, like high affinity, hydrophilicity, and high stability. The antibody of the invention can be provided in high concentrations liquid formulations with a viscosity suitable for ocular application. The antibody of the invention is suitable for the treatment of ocular vascular diseases.

### DESCRIPTION OF THE FIGURES

**Figure 1:** Schematic illustration of the Fab fragment of an anti-VEGF/anti-IL-1beta antibody of the invention. Shown is a top down view of a cognate VH/VL pair including the arrangement of CDR amino acid (upper image). VH domain is indicated in grey, VL domain is indicated in white. Furthermore, the spatial arrangement of the CDR regions is indicated. Paratope regions of an antibody of the invention is highlighted (lower image), with the VEGF paratope being arranged in the regions of H-CDR2, L-CDR1 and L-CDR2 and the IL-1beta paratope being arranged in the regions of H-CDR1, H-CDR3 and L-CDR2.

**Figure 2:** Amino acid sequences of VH domains of exemplary anti-VEGF/anti-IL-1beta antibodies of the invention. Kabat numbering of the amino acid position is indicated, as well as the CDR and FR regions. Amino acid positions contributing to the VEGF paratope, as well as the IL-1beta paratope as identified in Example 8 are highlighted.

**Figure 3:** Amino acid sequences of VL domains of exemplary anti-VEGF/anti-IL-1beta antibodies of the invention. Kabat numbering of the amino acid position is indicated, as well as the CDR and FR regions. Amino acid positions contributing to the VEGF paratope, as well as the IL-1beta paratope as identified in Example 8 are highlighted.

**Figure 4:** Simultaneous antigen binding of anti-VEGF/anti-IL-1beta antibody 1HVL12.85 to VEGF and IL-1beta as assessed via SPR according to Example 5.

**Figure 5:** Simultaneous antigen binding of anti-VEGF/anti-IL-1beta antibody RO7200394 to VEGF and IL-1beta as assessed via SPR according to Example 5.

**Figure 6:** Simultaneous antigen binding of prior art anti-VEGF/anti-IL-1beta antibody 0032 to VEGF and IL-1beta as assessed via SPR according to Example 5.

**Figure 7A:** Inhibition of binding of VEGF to hVEGFR2 in presence of antibody RO7200394 (Fab fragment) as assessed in Example 6. Receptor binding inhibition was assessed in presence and absence of the other target antigen of the bispecific antibody, IL-1beta.

5           **Figure 7B:** Inhibition of binding of VEGF to hVEGFR2 in presence of prior art antibody 0032 (full length IgG) as assessed in Example 6. Receptor binding inhibition was assessed in presence and absence of the other target antigen of the bispecific antibody, IL-1beta.

10           **Figure 8A:** Inhibition of binding of IL-1beta to IL-1betaR1 in presence of antibody RO7200394 (Fab fragment) as assessed in Example 6. Receptor binding inhibition was assessed in presence and absence of the other target antigen of the bispecific antibody, VEGF.

15           **Figure 8B:** Inhibition of binding of IL-1beta to IL-1betaR1 in presence of prior art antibody 0032 (full length IgG) as assessed in Example 6. Receptor binding inhibition was assessed in presence and absence of the other target antigen of the bispecific antibody, VEGF.

**Figure 9:** Competition ELISA assessing VEGF121-binding to VEGF-R1 in presence of indicated antibodies as assessed in Example 6

20           **Figure 10:** Competition ELISA assessing VEGF165-binding to VEGF-R1 in presence of indicated antibodies as assessed in Example 6

**Figure 11:** Results of Hydrophobic Interaction Chromatography (HIC) of antibodies of invention as assessed in Example 7

**Figure 12:** Results of Hydrophobic Interaction Chromatography (HIC) of prior art antibody 0032 (Example 7)

25           **Figure 13:** Viscosity of antibody 1HVL12.85 as assessed in Example 8

**Figure 14:** Viscosity of antibody RO7200394 as assessed in Example 8

## DETAILED DESCRIPTION OF THE INVENTION

### 1. Definitions

5 Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The methods and techniques of the present disclosure are generally  
10 performed according to conventional methods well known in the art. Generally, nomenclatures used in connection with, and techniques of biochemistry, enzymology, molecular, and cellular biology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art.

15 Unless otherwise defined herein the term “comprising of” shall include the term “consisting of”.

The term “about” as used herein in connection with a specific value (e.g. temperature, concentration, time and others) shall refer to a variation of +/- 1 % of the specific value that the term “about” refers to.

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

25 An “isolated” antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC) methods. For a review of methods for assessment of antibody purity, see, e.g., Flatman et al., J. Chromatogr. B 848:79-87  
30 (2007).

The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same

epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

The terms “full length antibody”, “intact antibody”, and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

The “class” of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. In certain embodiments, the antibody is of the IgG1 isotype. In certain embodiments, the antibody is of the IgG1 isotype with the P329G, L234A and L235A mutation to reduce Fc-region effector function. In other embodiments, the antibody is of the IgG2 isotype. In certain embodiments, the antibody is of the IgG4 isotype with the S228P mutation in the hinge region to improve stability of IgG4 antibody. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$ , respectively. The light chain of an antibody may be assigned to one of two types, called kappa ( $\kappa$ ) and lambda ( $\lambda$ ), based on the amino acid sequence of its constant domain.

The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

“Effector functions” refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

The term “variable region” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs) (see, e.g., Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007)). In the antibody of the invention, a single pair of a VH domain and a VL domain, i.e. a cognate VH/VL pair, specifically binds to its two targets: VEGF and IL-1beta.

A “DutaFab” is a bispecific antibody as disclosed in WO2012/163520. In a DutaFab a single pair of a VH domain and a VL domain specifically binds to two different epitopes, wherein one paratope comprises amino acid residues from from CDR-H2, CDR-L1 and CDR-L3 and the other paratope comprises amino residues from CDR-H1, CDR-H3 and CDR-L2. DutaFabs comprise two non-overlapping paratopes within a cognate VH/VL pair and may simultaneously bind to the two different epitopes. DutaFabs and methods for their generation by screening of libraries comprising monospecific Fab fragments are disclosed in WO2012/163520.

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

A “human consensus framework” is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup

is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., supra.

5 An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')<sub>2</sub>; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

10 A "paratope" or "antigen binding site", as used interchangeably herein, refers to a part of an antibody which recognizes and binds to an antigen. A paratope is formed by several individual amino acid residues from the antibody's heavy and light chain variable domains arranged that are arranged in spatial proximity in the tertiary structure of the Fv region. The antibodies of the invention comprise two "non-overlapping" paratopes in one cognate VH/VL pair. By "non-overlapping" is meant  
15 that none of the amino acids that are comprised in one of the two paratopes is comprised in the other paratope.

As used herein a "VEGF paratope" is a paratope or antigen binding site that binds to VEGF. The VEGF paratope of an antibody of the invention comprises amino acid residues from CDR-H2, CDR-L1 and CDR-L3 of the antibody.

20 As used herein an "IL-1beta paratope" is a paratope or antigen binding site that binds to IL-1beta. The IL-1betaparatope of an antibody of the invention comprises amino acid residues from CDR-H1, CDR-H3 and CDR-L2 of the antibody.

25 The term "VEGF", as used herein, refers to any native VEGF from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length", unprocessed VEGF as well as any form of VEGF that results from processing in the cell. The term also encompasses naturally occurring variants of VEGF, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human VEGF is shown in SEQ ID NO:26.

30 The terms "anti-VEGF antibody" and "an antibody that binds to VEGF" refer to an antibody that is capable of binding VEGF with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting VEGF. In one embodiment, the extent of binding of an anti-VEGF antibody to an unrelated, non-

VEGF protein is less than about 10% of the binding of the antibody to VEGF as measured, e.g., by surface plasmon resonance (SPR). In certain embodiments, an antibody that binds to VEGF has a dissociation constant ( $K_D$ ) of  $\leq 1$  nM,  $\leq 0.1$  nM, or  $\leq 0.01$  nM. An antibody is said to “specifically bind” to VEGF when the antibody  
5 has a  $K_D$  of  $1\mu\text{M}$  or less.

The term “IL-1beta”, as used herein, refers to any native IL-1beta from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses “full-length”, unprocessed IL-1beta as well as any form of IL-1beta that results from  
10 processing in the cell. The term also encompasses naturally occurring variants of IL-1beta, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human IL-1beta is shown in SEQ ID NO:27.

The terms “anti-IL-1beta antibody” and “an antibody that binds to anti-IL-1beta” refer to an antibody that is capable of binding anti-IL-1beta with sufficient affinity  
15 such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting anti-IL-1beta. In one embodiment, the extent of binding of an anti- anti-IL-1beta antibody to an unrelated, non- anti-IL-1beta protein is less than about 10% of the binding of the antibody to anti-IL-1beta as measured, e.g., by surface plasmon resonance (SPR). In certain embodiments, an antibody that binds to IL-1beta has a  
20 dissociation constant ( $K_D$ ) of  $\leq 1$  nM,  $\leq 0.1$  nM, or  $\leq 0.03$  nM. An antibody is said to “specifically bind” to anti-IL-1beta when the antibody has a  $K_D$  of  $1\mu\text{M}$  or less.

An antibody of the invention “simultaneously binds to human VEGF and human IL-1beta”, which means that (a) an antibody Fab fragment of the invention that is bound to human IL-1beta (also) specifically binds to human VEGF, and (b)  
25 an antibody Fab fragment of the invention that is bound to human VEGF (also) specifically binds to human IL-1beta. Simultaneous binding may be assessed with methods known in the art, e.g. by surface plasmon resonance as described herein.

The term “complementarity determining regions” or “CDRs” as used herein refers to each of the regions of an antibody variable domain which are hypervariable  
30 in sequence and contain antigen-contacting residues. Generally, antibodies comprise six CDRs: three in the VH domain (CDR-H1, CDR-H2, CDR-H3), and three in the VL domain (CDR-L1, CDR-L2, CDR-L3). Unless otherwise indicated, CDR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to the Kabat numbering system (Kabat et al., Sequences of Proteins

of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991).

“Framework” or “FR” as used herein refers to variable domain amino acid residues other than CDR residues. The framework of a variable domain generally consists of four framework domains: FR1, FR2, FR3, and FR4. Accordingly, the CDR and FR amino acid sequences generally appear in the following sequence in the (a) VH domain: FR1—CDR-H1—FR2—CDR-H2—FR3—CDR-H3—FR4; and (b) in the VL domain: FR1—CDR-L1—FR2—CDR-L2—FR3—CDR-L3—FR4.

According to the Kabat numbering system, as is used herein, framework and CDR regions are located at the following regions of the variable domains:

|    | <b>FR1</b> | <b>CDR-1</b> | <b>FR2</b> | <b>CDR2</b> | <b>FR3</b> | <b>CDR3</b> | <b>FR4</b> |
|----|------------|--------------|------------|-------------|------------|-------------|------------|
| VH | 1-30       | 31-35b*      | 36-49      | 50-65       | 66-94      | 95-102      | 103-113    |
| VL | 1-23       | 24-34        | 35-49      | 50-56       | 57-88      | 89-97       | 98-107     |

\* in CDR-H1 additional amino acids between position 35b and 36 may be present, herein referred to as positions “35c”, “35d” and “35e” as illustrated in **Figure 2**

The amino acid positions according to the Kabat numbering system referred to herein are illustrated in **Figure 2** in an alignment with the amino acid sequences of antibodies of the invention. References to amino acids at a certain position within the amino acid sequence are herein made as well known in the art by stating the respective amino acid and the amino acid position, e.g. “E2” refers to a glutamic acid residue located at Kabat position 2 of the amino acid sequence of the respective antibody domain.

“Affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant ( $K_D$ ). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described herein.

The term "epitope" denotes the site on an antigen, either proteinaceous or non-proteinaceous, to which an antibody binds. Epitopes can be formed both from contiguous amino acid stretches (linear epitope) or comprise non-contiguous amino acids (conformational epitope), e.g. coming in spatial proximity due to the folding of the antigen, i.e. by the tertiary folding of a proteinaceous antigen. Linear epitopes are typically still bound by an antibody after exposure of the proteinaceous antigen to denaturing agents, whereas conformational epitopes are typically destroyed upon treatment with denaturing agents. An epitope comprises at least 3, at least 4, at least 5, at least 6, at least 7, or 8-10 amino acids in a unique spatial conformation.

Screening for antibodies binding to a particular epitope (i.e., those binding to the same epitope) can be done using methods routine in the art such as, e.g., without limitation, alanine scanning, peptide blots (see Meth. Mol. Biol. 248 (2004) 443-463), peptide cleavage analysis, epitope excision, epitope extraction, chemical modification of antigens (see Prot. Sci. 9 (2000) 487-496), and cross-blocking (see "Antibodies", Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY).

Antigen Structure-based Antibody Profiling (ASAP), also known as Modification-Assisted Profiling (MAP), allows to bin a multitude of monoclonal antibodies specifically binding to VEGF or IL-1beta based on the binding profile of each of the antibodies from the multitude to chemically or enzymatically modified antigen surfaces (see, e.g., US 2004/0101920). The antibodies in each bin bind to the same epitope which may be a unique epitope either distinctly different from or partially overlapping with epitope represented by another bin.

Also competitive binding can be used to easily determine whether an antibody binds to the same epitope of VEGF or IL-1beta as, or competes for binding with, a reference antibody of the invention. For example, an "antibody that binds to the same epitopes on VEGF and IL-1beta" as a reference-antibody refers to an antibody that blocks binding of the reference-antibody to its antigens in respective competition assays by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in respective competition assays by 50% or more. Also for example, to determine if an antibody binds to the same epitope as a reference-antibody, the reference-antibody is allowed to bind to VEGF or IL-1beta under saturating conditions. After removal of the excess of the reference-antibody, the ability of an antibody in question to bind to VEGF or IL-1beta is assessed. If the antibody in question is able to bind to VEGF or IL-1beta after saturation binding of the reference-antibody, it can be concluded that the antibody in question binds to a

different epitope than the reference-antibody. But, if the antibody in question is not able to bind to VEGF or IL-1beta after saturation binding of the reference-antibody, then the antibody in question may bind to the same epitope as the epitope bound by the reference-antibody. To confirm whether the antibody in question binds to the same epitope or is just hampered from binding by steric reasons routine experimentation can be used (e.g., peptide mutation and binding analyses using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art). This assay should be carried out in two set-ups, i.e. with both of the antibodies being the saturating antibody. If, in both set-ups, only the first (saturating) antibody is capable of binding to VEGF or IL-1beta, then it can be concluded that the antibody in question and the reference-antibody compete for binding to VEGF or IL-1beta.

In some embodiments two antibodies are deemed to bind to the same or an overlapping epitope if a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50%, at least 75%, at least 90% or even 99% or more as measured in a competitive binding assay (see, e.g., Junghans et al., Cancer Res. 50 (1990) 1495-1502).

In some embodiments two antibodies are deemed to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody also reduce or eliminate binding of the other. Two antibodies are deemed to have "overlapping epitopes" if only a subset of the amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity for the purposes of the alignment. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, Clustal W, Megalign (DNASTAR) software or the FASTA program package. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length

of the sequences being compared. Alternatively, the percent identity values can be generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087 and is described in WO 2000/005319.

Unless otherwise indicated, For for purposes herein, however, % amino acid sequence identity values are generated using the ggsearch program of the FASTA package version 36.3.8c or later with a BLOSUM50 comparison matrix. The FASTA program package was authored by W. R. Pearson and D. J. Lipman (1988), "Improved Tools for Biological Sequence Analysis", PNAS 85:2444-2448; W. R. Pearson (1996) "Effective protein sequence comparison" Meth. Enzymol. 266:227-258; and Pearson et. al. (1997) Genomics 46:24-36 and is publicly available from [www.fasta.bioch.virginia.edu/fasta\\_www2/fasta\\_down.shtml](http://www.fasta.bioch.virginia.edu/fasta_www2/fasta_down.shtml) or [www.ebi.ac.uk/Tools/sss/fasta](http://www.ebi.ac.uk/Tools/sss/fasta). Alternatively, a public server accessible at [fasta.bioch.virginia.edu/fasta\\_www2/index.cgi](http://fasta.bioch.virginia.edu/fasta_www2/index.cgi) can be used to compare the sequences, using the ggsearch (global protein:protein) program and default options (BLOSUM50; open: -10; ext: -2; Ktup = 2) to ensure a global, rather than local, alignment is performed. Percent amino acid identity is given in the output alignment header.

The term "nucleic acid molecule" or "polynucleotide" includes any compound and/or substance that comprises a polymer of nucleotides. Each nucleotide is composed of a base, specifically a purine- or pyrimidine base (i.e. cytosine (C), guanine (G), adenine (A), thymine (T) or uracil (U)), a sugar (i.e. deoxyribose or ribose), and a phosphate group. Often, the nucleic acid molecule is described by the sequence of bases, whereby said bases represent the primary structure (linear structure) of a nucleic acid molecule. The sequence of bases is typically represented from 5' to 3'. Herein, the term nucleic acid molecule encompasses deoxyribonucleic acid (DNA) including e.g. complementary DNA (cDNA) and genomic DNA, ribonucleic acid (RNA), in particular messenger RNA (mRNA), synthetic forms of DNA or RNA, and mixed polymers comprising two or more of these molecules. The nucleic acid molecule may be linear or circular. In addition, the term nucleic acid molecule includes both, sense and antisense strands, as well as single stranded and double stranded forms. Moreover, the herein described nucleic acid molecule can contain naturally occurring or non-naturally occurring nucleotides. Examples of non-naturally occurring nucleotides include modified

nucleotide bases with derivatized sugars or phosphate backbone linkages or chemically modified residues. Nucleic acid molecules also encompass DNA and RNA molecules which are suitable as a vector for direct expression of an antibody of the invention in vitro and/or in vivo, e.g. in a host or patient. Such DNA (e.g. cDNA) or RNA (e.g. mRNA) vectors, can be unmodified or modified. For example, mRNA can be chemically modified to enhance the stability of the RNA vector and/or expression of the encoded molecule so that mRNA can be injected into a subject to generate the antibody in vivo (see e.g. Stadler et al, Nature Medicine 2017, published online 12 June 2017, doi:10.1038/nm.4356 or EP 2 101 823 B1).

10           An “isolated” nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

15           “Isolated nucleic acid encoding” an antibody refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

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

25           The terms “host cell”, “host cell line”, and “host cell culture” are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells”, which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

30           The term “pharmaceutical composition” or “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of an

active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the pharmaceutical composition would be administered.

5 A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical composition or formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

10 An “effective amount” of an agent, e.g., a pharmaceutical composition, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

An “individual” or “subject” is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

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

25 The term “ocular disease,” as used herein, includes any ocular disease associated with pathological angiogenesis and/or atrophy. An ocular disease may be characterized by altered or unregulated proliferation and/or invasion of new blood vessels into the structures of ocular tissues such as the retina or cornea. An ocular disease may be characterized by atrophy of retinal tissue (photoreceptors and the  
30 underlying retinal pigment epithelium (RPE) and choriocapillaris). Non-limiting ocular diseases include, for example, AMD (e.g., wet AMD, dry AMD, intermediate AMD, advanced AMD, and geographic atrophy (GA)), macular degeneration, macular edema, DME (e.g., focal, non-center DME and diffuse, center-involved DME), retinopathy, diabetic retinopathy (DR) (e.g., proliferative DR (PDR), non-

proliferative DR (NPDR), and high-altitude DR), other ischemia-related retinopathies, ROP, retinal vein occlusion (RVO) (e.g., central (CRVO) and branched (BRVO) forms), CNV (e.g., myopic CNV), corneal neovascularization, diseases associated with corneal neovascularization, retinal neovascularization, diseases associated with retinal/choroidal neovascularization, central serous retinopathy (CSR), pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, FEVR, Coats' disease, Norrie Disease, retinal abnormalities associated with osteoporosis-pseudoglioma syndrome (OPPG), subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, retinitis pigmentosa (RP), hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, cystoid macular edema (CME), vasculitis, papilloedema, retinitis, including but not limited to CMV retinitis, ocular melanoma, retinal blastoma, conjunctivitis (e.g., infectious conjunctivitis and non-infectious (e.g., allergic) conjunctivitis), Leber congenital amaurosis (also known as Leber's congenital amaurosis or LCA), uveitis (including infectious and non-infectious uveitis), choroiditis (e.g., multifocal choroiditis), ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, Sjögren's disease, and other ophthalmic diseases wherein the disease or disease is associated with ocular neovascularization, vascular leakage, and/or retinal edema or retinal atrophy. Additional exemplary ocular diseases include retinoschisis (abnormal splitting of the retina neurosensory layers), diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue, including all forms of proliferative vitreoretinopathy. Exemplary diseases associated with corneal neovascularization include, but are not limited to, epidemic keratoconjunctivitis, vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, terygium keratitis sicca, Sjögren's syndrome, acne rosacea, phlyctenulosis, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, Herpes simplex infections, Herpes zoster infections, protozoan infections, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegener's sarcoidosis, scleritis, Stevens-Johnson syndrome, periphigoid radial keratotomy, and corneal graft rejection. Exemplary diseases associated with choroidal neovascularization and defects in the retina vasculature, including increased vascular leak, aneurisms and capillary drop-out include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Paget's disease, vein occlusion, artery

occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, retina edema (including macular edema), Eales disease, Behcet's disease, infections causing retinitis or choroiditis (e.g., multifocal choroiditis), presumed ocular histoplasmosis, Best's disease (vitelliform macular degeneration), myopia, optic pits, pars planitis, retinal detachment (e.g., chronic retinal detachment), hyperviscosity syndromes, toxoplasmosis, trauma, and post-laser complications. Exemplary diseases associated with atrophy of retinal tissues (photoreceptors and the underlying RPE) include, but are not limited to, atrophic or nonexudative AMD (e.g., geographic atrophy or advanced dry AMD), macular atrophy (e.g., atrophy associated with neovascularization and/or geographic atrophy), diabetic retinopathy, Stargardt's disease, Sorsby Fundus Dystrophy, retinoschisis and retinitis pigmentosa.

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

## **2. Detailed description of the embodiments of the invention**

In one aspect, the invention is based, in part, on the provision of bispecific antibodies for therapeutic application. In certain aspects, antibodies that bind to human VEGF and human IL-1beta are provided. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of vascular diseases, e.g. ocular vascular diseases.

### **A. Exemplary antibodies that bind to human VEGF and human IL-1beta**

In one aspect, the invention provides antibodies that bind to human VEGF and human IL-1beta. In one aspect, provided are isolated antibodies that bind to human VEGF and human IL-1beta. In one aspect, the invention provides antibodies that specifically bind to human VEGF and human IL-1beta.

In certain aspects, an antibody that binds to human VEGF and to human IL-1beta is provided, wherein the antibody comprises a VEGF paratope (i.e. an antigen binding site that binds to VEGF) and an IL-1beta paratope (i.e. an antigen binding site that binds to IL-1beta) within one cognate pair of a VL domain and a VH domain, wherein

- the VEGF paratope comprises amino acid residues from CDR-H2, CDR-L1 and CDR-L3 of the antibody, wherein the IL-1beta paratope comprises amino acid residues from the CDR-H1, CDR-H3 and CDR-L2 of the antibody; and/or
- 5 • the pair of the variable light chain domain and the variable heavy chain domain simultaneously binds to human VEGF and human IL-1beta; and/or
- none of the amino acids that are comprised in the VEGF paratope are comprised in the IL-1beta paratope; and/or
- 10 • none of the amino acids that are comprised in the IL-1beta paratope are comprised in the VEGF paratope; and/or
- the antibody binds to the same epitope on human VEGF and to the same epitope on human IL-1beta as an antibody with a variable heavy chain domain of SEQ ID NO: 11 and a variable light chain domain of SEQ ID NO: 12; and/or
- 15 • an antibody Fab fragment of the antibody binds (i) to human VEGF121 with a  $K_D$  of less than 10 pM as measured by surface plasmon resonance, and (ii) to human IL-1beta with a  $K_D$  of less than 30 pM as measured by surface plasmon resonance; and/or
- an antibody Fab fragment of the antibody exhibits an aggregation onset temperature of more than 70 °C; and/or
- 20 • an antibody Fab fragment of the antibody exhibits a melting temperature of more than 80 °C as measured by dynamic light scattering; and/or
- binding of an antibody Fab fragment of the antibody to human VEGF inhibits binding of VEGF to VEGFR2 with an  $IC_{50}$  of less than 50 nM as measured by surface plasmon resonance; and wherein binding of an antibody Fab fragment of the antibody to human IL-1beta inhibits binding of IL-1beta to IL-1betaR1 with an  $IC_{50}$  of less than 30 nM as measured by surface plasmon resonance.
- 25

In another aspect, the invention provides an antibody comprising a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8.

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In another aspect, the invention provides an antibody comprising a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system

In another aspect, the invention provides an antibody comprising a VH domain comprising amino acid residues E2, G26, V28, K30, W31, N35b, D35c, K52a, D55, H56, Y58, T61, K62, F63, I64, R66, R83, K94, D95, V96, F98, and D101, and a VL domain comprising amino acid residues I2, Y27, W27a, S27c, S27d, L32, Y49, D50, Y53, K54, L56, G57, E67, D68, Q69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the VH and VL domains is according to the Kabat numbering system. In one embodiment the antibody comprises a VEGF paratope comprising the following amino acid residues in the VH domain: D55, H56, Y58, T61, K62, F63, I64, R66, and R83, and the following amino acid residues in the VL domain: I2, Y27, W27a, S27c, S27d, E67, D68, Q69, R92, Y93, H94, and Y96; and an IL-1beta paratope comprising the following amino acid residues in the VH domain :E2, G26, V28, K30, W31, N35b, D35c, K52a, K94, D95, V96, F98, and D101, and the following amino acid residues in the VL domain: L32, Y49, D50, Y53, K54, L56, G57, Y91.

In another aspect, the invention provides an antibody comprising (a) a VH domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:12.

In another aspect, the invention provides an antibody comprising (a) a VH domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:11, wherein the VH domain comprises amino acid residues E2, G26, V28, K30, W31, N35b, D35c, K52a, D55, H56, Y58, T61, K62, F63, I64, R66, R83, K94, D95, V96, F98, and D101; and (b) a VL domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:12, wherein the VL domain comprises amino acid residues I2, Y27, W27a, S27c, S27d, L32, Y49, D50, Y53, K54, L56, G57, E67, D68, Q69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.

In another aspect, the invention provides an antibody comprising a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:12.

In another aspect, the invention provides an antibody comprising a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:11, wherein the VH domain comprises amino acid residues E2, G26, V28, K30, R66, R83, and K94; and (b) a VL domain comprising

an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:12, wherein the VL domain comprises amino acid residues I2, Y49, G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.

In another aspect, the invention provides an antibody comprising a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94, and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, comprising (a) a VH domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:12.

In another aspect, the invention provides an antibody comprising (a) a VH domain comprising an amino acid sequence of SEQ ID NO:11 with 1 to 15, 1 to 10, or 1 to 5 amino acid substitutions; and (b) a variable light chain domain comprising an amino acid sequence of SEQ ID NO:12 with 1 to 15, 1 to 10, or 1 to 5 amino acid substitutions.

In another aspect, the invention provides an antibody comprising comprising (a) a VH domain comprising an amino acid sequence of SEQ ID NO:11 with 1 to 15, 1 to 10, or 1 to 5 amino acid substitutions, wherein the amino acid substitutions are located at positions 3 to 25, 36 to 49, 97 to 82c, 84 to 93, or 103 to 113 of SEQ ID NO:11; and (b) a variable light chain domain comprising an amino acid sequence of SEQ ID NO:12 with 1 to 15, 1 to 10, or 1 to 5 amino acid substitutions, wherein the amino acid substitutions are located at positions 1, 4, 6, 8 to 23, 35 to 48, 58 to

66, 70 to 88, or 98 to 107 of SEQ ID NO:12, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.

5 In another aspect, the invention provides an antibody comprising a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain  
10 comprising an amino acid sequence of SEQ ID NO:11 with 1 to 15, 1 to 10, or 1 to 5 amino acid substitutions; and (b) a variable light chain domain comprising an amino acid sequence of SEQ ID NO:12 with 1 to 15, 1 to 10, or 1 to 5 amino acid substitutions.

15 In another aspect, the invention provides an antibody comprising a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain  
20 comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the  
25 Kabat numbering system, and comprising (a) a VH domain comprising an amino acid sequence of SEQ ID NO:11 with 1 to 15, 1 to 10, or 1 to 5 amino acid substitutions; and (b) a variable light chain domain comprising an amino acid sequence of SEQ ID NO:12 with 1 to 15, 1 to 10, or 1 to 5 amino acid substitutions.

30 In one aspect, the invention provides an antibody comprising a VH domain having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:11. In certain aspects, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions,  
35 or deletions relative to the reference sequence, but an antibody that binds to human

VEGF and human IL-1beta comprising that sequence retains the ability to bind to human VEGF and human IL-1beta. In certain aspects, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:11. In certain aspects, substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs). In a particular aspect, the VH comprises (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15.

In one aspect, the invention provides an antibody comprising a VL domain having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:12. In certain aspects, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an antibody that binds to human VEGF and human IL-1beta comprising that sequence retains the ability to bind to human VEGF and human IL-1beta. In certain aspects, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:12. In certain aspects, substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs). In a particular aspect, the VL comprises (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8.

In another aspect, an antibody that binds to human VEGF and human IL-1beta is provided, wherein the antibody comprises a VH sequence as in any of the aspects provided above, and a VL sequence as in any of the aspects provided above. In one aspect, the antibody comprises the VH and VL sequences in SEQ ID NO:11 and SEQ ID NO:12, respectively, including post-translational modifications of those sequences.

In another aspect, an antibody that binds to human VEGF and human IL-1beta is provided, wherein the antibody comprises a heavy chain amino acid sequence of SEQ ID NO:20 and a light chain amino acid sequence of SEQ ID NO:19.

In another aspect, an antibody that binds to human VEGF and human IL-1beta is provided, wherein the antibody comprises a heavy chain amino acid

sequence of SEQ ID NO:18 and a light chain amino acid sequence of SEQ ID NO:19.

In a further aspect of the invention, an antibody that binds to human VEGF and human IL-1beta according to any of the above aspects is a monoclonal antibody.

5 In one aspect, an antibody that binds to human VEGF and human IL-1beta is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')<sub>2</sub> fragment. In another aspect, the antibody is a full length antibody.

In a further aspect, an antibody that binds to human VEGF and human IL-1beta according to any of the above aspects may incorporate any of the features, singly or  
10 in combination, as described in Sections 1-7 below:

### 1. Antibody Affinity

In certain embodiments, an antibody provided herein binds to VEGF with a dissociation constant ( $K_D$ ) of  $\leq 1$  nM,  $\leq 0.1$  nM, or  $\leq 0.01$  nM. In certain  
15 embodiments, an antibody that binds to IL-1beta has a dissociation constant ( $K_D$ ) of  $\leq 1$  nM,  $\leq 0.1$  nM, or  $\leq 0.03$  nM.

In one aspect,  $K_D$  is measured using a BIAcore® surface plasmon resonance assay.

For example, the  $K_D$  of antibody binding to VEGF is measured in an assay using a BIAcore®-2000 or a BIAcore®-3000 (BIAcore, Inc., Piscataway, NJ)  
20 performed at 25°C with immobilized VEGF121 on C1 chips at ~10 response units (RU). For kinetics measurements, two-fold serial dilutions of Fab (1.2 – 100 nM) are injected in HBS-P+ (10 mM HEPES, 150 mM NaCl pH 7.4, 0.05% Surfactant P20) at 25°C at a flow rate of approximately 30 µl/min. Association rates ( $k_{on}$ ) and dissociation rates ( $k_{off}$ ) are calculated using a simple one-to-one Langmuir binding  
25 model (BIAcore® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant ( $K_D$ ) is calculated as the ratio  $k_{off}/k_{on}$ . See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999).

For example, the  $K_D$  of antibody binding to IL-1beta is measured in an assay using a BIAcore®-2000 or a BIAcore®-3000 (BIAcore, Inc., Piscataway, NJ)  
30 performed at 25°C with immobilized bispecific antibody on C1 chips at ~20 response units (RU). For kinetics measurements, two-fold serial dilutions of human IL-1beta (0.74 to 60 nM) are injected in HBS-P+ (10 mM HEPES, 150 mM NaCl pH 7.4,

0.05% Surfactant P20) at 25°C at a flow rate of approximately 30 µl/min. Association rates ( $k_{on}$ ) and dissociation rates ( $k_{off}$ ) are calculated using a simple one-to-one Langmuir binding model (BIAcore® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant ( $K_D$ ) is calculated as the ratio  $k_{off}/k_{on}$ . See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999).

## 2. Antibody Fragments

In certain aspects, an antibody provided herein is an antibody fragment.

In one aspect, the antibody fragment is a Fab, Fab', Fab'-SH, or F(ab')<sub>2</sub> fragment, in particular a Fab fragment. Papain digestion of intact antibodies produces two identical antigen-binding fragments, called "Fab" fragments containing each the heavy- and light-chain variable domains (VH and VL, respectively) and also the constant domain of the light chain (CL) and the first constant domain of the heavy chain (CH1). The term "Fab fragment" thus refers to an antibody fragment comprising a light chain comprising a VL domain and a CL domain, and a heavy chain fragment comprising a VH domain and a CH1 domain. "Fab' fragments" differ from Fab fragments by the addition of residues at the carboxy terminus of the CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH are Fab' fragments in which the cysteine residue(s) of the constant domains bear a free thiol group. Pepsin treatment yields an F(ab')<sub>2</sub> fragment that has two antigen-binding sites (two Fab fragments) and a part of the Fc region. For discussion of Fab and F(ab')<sub>2</sub> fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as recombinant production by recombinant host cells (e.g., E. coli, CHO), as described herein.

## 3. Thermal stability

Antibodies provided herein exhibit superior thermal stability. In certain embodiments, a Fab fragment of an antibody provided herein exhibits an aggregation onset temperature of more than 70 °C. In certain embodiments, a Fab fragment of an antibody provided herein exhibits a melting temperature of more than 80 °C as measured by dynamic light scattering.

#### 4. Library-Derived Antibodies

In certain aspects, an antibody provided herein is derived from a library. Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. Methods for screening combinatorial libraries are reviewed, e.g., in Lerner et al. in *Nature Reviews* 16:498-508 (2016). For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Frenzel et al. in *mAbs* 8:1177-1194 (2016); Bazan et al. in *Human Vaccines and Immunotherapeutics* 8:1817-1828 (2012) and Zhao et al. in *Critical Reviews in Biotechnology* 36:276-289 (2016) as well as in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and in Marks and Bradbury in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al. in *Annual Review of Immunology* 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al. in *EMBO Journal* 12: 725-734 (1993). Furthermore, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter in *Journal of Molecular Biology* 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent Nos. 5,750,373; 7,985,840; 7,785,903 and 8,679,490 as well as US Patent Publication Nos. 2005/0079574, 2007/0117126, 2007/0237764 and 2007/0292936.

Further examples of methods known in the art for screening combinatorial libraries for antibodies with a desired activity or activities include ribosome and mRNA display, as well as methods for antibody display and selection on bacteria,

mammalian cells, insect cells or yeast cells. Methods for yeast surface display are reviewed, e.g., in Scholler et al. in *Methods in Molecular Biology* 503:135-56 (2012) and in Cherf et al. in *Methods in Molecular biology* 1319:155-175 (2015) as well as in Zhao et al. in *Methods in Molecular Biology* 889:73-84 (2012). Methods for  
5 ribosome display are described, e.g., in He et al. in *Nucleic Acids Research* 25:5132-5134 (1997) and in Hanes et al. in *PNAS* 94:4937-4942 (1997).

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

## 5. Multispecific Antibodies

10 In certain aspects, an antibody provided herein is a multispecific antibody. "Multispecific antibodies" are monoclonal antibodies that have binding specificities for at least two different sites, i.e., different epitopes on different antigens or different epitopes on the same antigen. In certain aspects, the multispecific antibody has three or more binding specificities.

15 Multispecific antibodies with three or more binding specificities comprising antibodies provided herein may be provided in an asymmetric form with a domain crossover in one or more binding arms of the same antigen specificity, i.e. by exchanging the VH/VL domains (see e.g., WO 2009/080252 and WO 2015/150447), the CH1/CL domains (see e.g., WO 2009/080253) or the complete Fab arms (see  
20 e.g., WO 2009/080251, WO 2016/016299, also see Schaefer et al, *PNAS*, 108 (2011) 1187-1191, and Klein et al., *MAbs* 8 (2016) 1010-20). Various further molecular formats for multispecific antibodies are known in the art and are included herein (see e.g., Spiess et al., *Mol Immunol* 67 (2015) 95-106).

## 6. Antibody Variants

25 In certain aspects, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to alter the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such  
30 modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final

construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

In certain aspects, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the CDRs and FRs. Conservative substitutions are shown in the Table below under the heading of “preferred substitutions”. More substantial changes are provided in Table 1 under the heading of “exemplary substitutions”, and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

**TABLE**

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

| Original Residue | Exemplary Substitutions             | Preferred Substitutions |
|------------------|-------------------------------------|-------------------------|
| Val (V)          | Ile; Leu; Met; Phe; Ala; Norleucine | Leu                     |

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;
- 5 (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

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

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

Alterations (e.g., substitutions) may be made in CDRs, e.g., to improve antibody affinity. Such alterations may be made in CDR “hotspots”, i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)),  
25 and/or residues that contact antigen, with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O’Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some aspects of affinity maturation, diversity is introduced into the variable genes  
30 chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the

desired affinity. Another method to introduce diversity involves CDR-directed approaches, in which several CDR residues (e.g., 4-6 residues at a time) are randomized. CDR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

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

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

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

a) Glycosylation variants

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

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

In one aspect, antibody variants are provided having a non-fucosylated oligosaccharide, i.e. an oligosaccharide structure that lacks fucose attached (directly or indirectly) to an Fc region. Such non-fucosylated oligosaccharide (also referred to as “afucosylated” oligosaccharide) particularly is an N-linked oligosaccharide which lacks a fucose residue attached to the first GlcNAc in the stem of the biantennary oligosaccharide structure. In one aspect, antibody variants are provided having an increased proportion of non-fucosylated oligosaccharides in the Fc region as compared to a native or parent antibody. For example, the proportion of non-fucosylated oligosaccharides may be at least about 20%, at least about 40%, at least about 60%, at least about 80%, or even about 100% (i.e. no fucosylated oligosaccharides are present). The percentage of non-fucosylated oligosaccharides is the (average) amount of oligosaccharides lacking fucose residues, relative to the sum of all oligosaccharides attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2006/082515, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about  $\pm 3$  amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such antibodies having an increased proportion of non-fucosylated oligosaccharides in the Fc region may have improved Fc $\gamma$ RIIIa receptor binding

and/or improved effector function, in particular improved ADCC function. See, e.g., US 2003/0157108; US 2004/0093621.

5 Examples of cell lines capable of producing antibodies with reduced fucosylation include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US 2003/0157108; and WO 2004/056312, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87:614-622 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO 2003/085107), or cells with reduced or abolished  
10 activity of a GDP-fucose synthesis or transporter protein (see, e.g., US2004259150, US2005031613, US2004132140, US2004110282).

In a further aspect, antibody variants are provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have  
15 reduced fucosylation and/or improved ADCC function as described above. Examples of such antibody variants are described, e.g., in Umana et al., *Nat Biotechnol* 17, 176-180 (1999); Ferrara et al., *Biotechnol Bioeng* 93, 851-861 (2006); WO 99/54342; WO 2004/065540, WO 2003/011878.

Antibody variants with at least one galactose residue in the oligosaccharide  
20 attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087; WO 1998/58964; and WO 1999/22764.

#### **b) Fc region variants**

In certain aspects, one or more amino acid modifications may be introduced  
25 into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> or IgG<sub>4</sub> Fc region) comprising an amino acid modification (e.g., a substitution) at one or more amino acid positions.

In certain aspects, the invention contemplates an antibody variant that  
30 possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC)) are unnecessary or deleterious. *In*

*vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g., Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006); WO 2013/120929 A1).

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

Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

5 In certain aspects, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

10 In certain aspects, an antibody variant comprises an Fc region with one or more amino acid substitutions which diminish Fc $\gamma$ R binding, e.g., substitutions at positions 234 and 235 of the Fc region (EU numbering of residues). In one aspect, the substitutions are L234A and L235A (LALA). In certain aspects, the antibody variant further comprises D265A and/or P329G in an Fc region derived from a human IgG<sub>1</sub> Fc region. In one aspect, the substitutions are L234A, L235A and P329G (LALA-PG) in an Fc region derived from a human IgG<sub>1</sub> Fc region. (See, e.g., WO 2012/130831). In another aspect, the substitutions are L234A, L235A and D265A  
15 (LALA-DA) in an Fc region derived from a human IgG<sub>1</sub> Fc region.

In some aspects, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

20 Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region  
25 to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 252, 254, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (See, e.g., US Patent No. 7,371,826; Dall'Acqua, W.F., et al. *J. Biol. Chem.* 281 (2006) 23514-23524).

30 Fc region residues critical to the mouse Fc-mouse FcRn interaction have been identified by site-directed mutagenesis (see e.g. Dall'Acqua, W.F., et al. *J. Immunol* 169 (2002) 5171-5180). Residues I253, H310, H433, N434, and H435 (EU index numbering) are involved in the interaction (Medesan, C., et al., *Eur. J. Immunol.* 26 (1996) 2533; Firan, M., et al., *Int. Immunol.* 13 (2001) 993; Kim, J.K., et al., *Eur. J.*

Immunol. 24 (1994) 542). Residues I253, H310, and H435 were found to be critical for the interaction of human Fc with murine FcRn (Kim, J.K., et al., Eur. J. Immunol. 29 (1999) 2819). Studies of the human Fc-human FcRn complex have shown that residues I253, S254, H435, and Y436 are crucial for the interaction (Firan, M., et al.,  
5 Int. Immunol. 13 (2001) 993; Shields, R.L., et al., J. Biol. Chem. 276 (2001) 6591-6604). In Yeung, Y.A., et al. (J. Immunol. 182 (2009) 7667-7671) various mutants of residues 248 to 259 and 301 to 317 and 376 to 382 and 424 to 437 have been reported and examined.

In certain aspects, an antibody variant comprises an Fc region with one or more  
10 amino acid substitutions, which reduce FcRn binding, e.g., substitutions at positions 253, and/or 310, and/or 435 of the Fc-region (EU numbering of residues). In certain aspects, the antibody variant comprises an Fc region with the amino acid substitutions at positions 253, 310 and 435. In one aspect, the substitutions are I253A, H310A and H435A in an Fc region derived from a human IgG1 Fc-region.  
15 See, e.g., Grevys, A., et al., J. Immunol. 194 (2015) 5497-5508.

In certain aspects, an antibody variant comprises an Fc region with one or more amino acid substitutions, which reduce FcRn binding, e.g., substitutions at positions 310, and/or 433, and/or 436 of the Fc region (EU numbering of residues). In certain aspects, the antibody variant comprises an Fc region with the amino acid  
20 substitutions at positions 310, 433 and 436. In one aspect, the substitutions are H310A, H433A and Y436A in an Fc region derived from a human IgG1 Fc-region. (See, e.g., WO 2014/177460 A1).

In certain aspects, an antibody variant comprises an Fc region with one or more amino acid substitutions which increase FcRn binding, e.g., substitutions at positions  
25 252, and/or 254, and/or 256 of the Fc region (EU numbering of residues). In certain aspects, the antibody variant comprises an Fc region with amino acid substitutions at positions 252, 254, and 256. In one aspect, the substitutions are M252Y, S254T and T256E in an Fc region derived from a human IgG1 Fc-region. See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No.  
30 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

The C-terminus of the heavy chain of the antibody as reported herein can be a complete C-terminus ending with the amino acid residues PGK. The C-terminus of the heavy chain can be a shortened C-terminus in which one or two of the C  
terminal amino acid residues have been removed. In one preferred aspect, the C-  
35 terminus of the heavy chain is a shortened C-terminus ending PG. In one aspect of

all aspects as reported herein, an antibody comprising a heavy chain including a C-terminal CH3 domain as specified herein, comprises the C-terminal glycine-lysine dipeptide (G446 and K447, EU index numbering of amino acid positions). In one aspect of all aspects as reported herein, an antibody comprising a heavy chain  
5 including a C-terminal CH3 domain, as specified herein, comprises a C-terminal glycine residue (G446, EU index numbering of amino acid positions).

**c) Cysteine engineered antibody variants**

In certain aspects, it may be desirable to create cysteine engineered antibodies, e.g., THIOMAB™ antibodies, in which one or more residues of an antibody are  
10 substituted with cysteine residues. In particular aspects, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein.  
15 Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541, 8,30,930, 7,855,275, 9,000,130, or WO 2016040856.

**7. Immunoconjugates**

The invention also provides immunoconjugates comprising an antibody that binds to human VEGF and human IL-1beta as disclosed herein conjugated  
20 (chemically bonded) to one or more therapeutic agents such as cytotoxic agents, chemotherapeutic agents, drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one aspect, an immunoconjugate is an antibody-drug conjugate (ADC) in  
25 which an antibody is conjugated to one or more of the therapeutic agents mentioned above. The antibody is typically connected to one or more of the therapeutic agents using linkers. An overview of ADC technology including examples of therapeutic agents and drugs and linkers is set forth in Pharmacol Review 68:3-19 (2016).

**B. Recombinant Methods and Compositions**

30 Antibodies may be produced using recombinant methods and compositions, e.g., as described in US 4,816,567. For these methods one or more isolated nucleic acid(s) encoding an antibody are provided.

In one aspect, isolated nucleic acids encoding an antibody of the invention are provided.

5 In one aspect, a method of making an antibody that binds to human VEGF and human IL-1beta is provided, wherein the method comprises culturing a host cell comprising nucleic acid(s) encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

10 For recombinant production of an antibody that binds to human VEGF and human IL-1beta, nucleic acids encoding the antibody, e.g., as described above, are isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acids may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody) or produced by recombinant methods or obtained by chemical synthesis.

15 Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., US 5,648,237, US 5,789,199, and US 5,840,523. (See also  
20 Charlton, K.A., In: Methods in Molecular Biology, Vol. 248, Lo, B.K.C. (ed.), Humana Press, Totowa, NJ (2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

25 Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293T cells as described, e.g., in Graham, F.L. et al., J. Gen Virol. 36 (1977) 59-74); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, J.P., Biol. Reprod. 23 (1980)  
30 243-252); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells (as described, e.g., in Mather, J.P. et al., Annals N.Y. Acad. Sci. 383 (1982) 44-68); MRC 5 cells; and FS4 cells. Other  
35 useful mammalian host cell lines include Chinese hamster ovary (CHO) cells,

including DHFR- CHO cells (Urlaub, G. et al., Proc. Natl. Acad. Sci. USA 77 (1980) 4216-4220); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki, P. and Wu, A.M., Methods in Molecular Biology, Vol. 248, Lo, B.K.C. (ed.),  
5 Humana Press, Totowa, NJ (2004), pp. 255-268.

In one aspect, the host cell is eukaryotic, e.g., a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell).

### C. Pharmaceutical Compositions

10 In a further aspect, provided are pharmaceutical compositions comprising any of the antibodies provided herein, e.g., for use in any of the below therapeutic methods. In one aspect, a pharmaceutical composition comprises any of the antibodies provided herein and a pharmaceutically acceptable carrier. In another aspect, a pharmaceutical composition comprises any of the antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

15 Pharmaceutical compositions of an antibody that binds to human VEGF and human IL-1beta as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized compositions or aqueous solutions. Pharmaceutically  
20 acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as histidine, phosphate, citrate, acetate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride;  
25 benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine,  
30 arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein  
35 further include interstitial drug dispersion agents such as soluble neutral-active

hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX<sup>®</sup>, Halozyme, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody compositions are described in US Patent No. 6,267,958. Aqueous antibody compositions include those described in US Patent No. 6,171,586 and WO 2006/044908, the latter compositions including a histidine-acetate buffer.

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

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Pharmaceutical compositions for sustained-release may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules.

The pharmaceutical compositions to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

#### **D. Therapeutic Methods and Routes of Administration**

Any of the antibodies that bind to human VEGF and human IL-1beta provided herein may be used in therapeutic methods.

In one aspect, an antibody that binds to human VEGF and human IL-1beta for use as a medicament is provided. In further aspects, an antibody that binds to human VEGF and human IL-1beta for use in treating a vascular disease is provided. In certain aspects, an antibody that binds to human VEGF and human IL-1beta for use in a method of treatment is provided. In certain aspects, the invention provides an antibody that binds to human VEGF and human IL-1beta for use in a method of treating an individual having a vascular disease comprising administering to the individual an effective amount of the antibody that binds to human VEGF and human IL-1beta. In one such aspect, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent (e.g., one, two, three, four, five, or six additional therapeutic agents), e.g., as described below. In further aspects, the invention provides an antibody that binds to human VEGF and human IL-1beta for use in inhibiting angiogenesis. In certain aspects, the invention provides an antibody that binds to human VEGF and human IL-1beta for use in a method inhibiting angiogenesis in an individual comprising administering to the individual an effective amount of the antibody that binds to human VEGF and human IL-1beta to inhibit angiogenesis. An "individual" according to any of the above aspects is preferably a human.

In further aspects, an antibody that binds to human VEGF and human IL-1beta for use in treating an ocular disease is provided. In one embodiment the ocular disease is selected from AMD (in one embodiment wet AMD, dry AMD, intermediate AMD, advanced AMD, and geographic atrophy (GA)), macular degeneration, macular edema, DME (in one embodiment focal, non-center DME and diffuse, center-involved DME), retinopathy, diabetic retinopathy (DR) (in one embodiment proliferative DR (PDR), non-proliferative DR (NPDR), and high-altitude DR), other ischemia-related retinopathies, ROP, retinal vein occlusion (RVO) (in one embodiment central (CRVO) and branched (BRVO) forms), CNV (in one embodiment myopic CNV), corneal neovascularization, diseases associated with corneal neovascularization, retinal neovascularization, diseases associated with retinal/choroidal neovascularization, central serous retinopathy (CSR), pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, FEVR, Coats' disease, Norrie Disease, retinal abnormalities associated with osteoporosis-pseudoglioma syndrome (OPPG), subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, retinitis pigmentosa (RP), hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, cystoid macular edema (CME), vasculitis, papilloedema, retinitis, including but not limited

to CMV retinitis, ocular melanoma, retinal blastoma, conjunctivitis (in one embodiment infectious conjunctivitis and non-infectious (in one embodiment allergic) conjunctivitis), Leber congenital amaurosis (also known as Leber's congenital amaurosis or LCA), uveitis (including infectious and non-infectious uveitis), choroiditis (in one embodiment multifocal choroiditis), ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, Sjögren's disease, and other ophthalmic diseases wherein the disease or disease is associated with ocular neovascularization, vascular leakage, and/or retinal edema or retinal atrophy. In one embodiment the ocular disease is selected from AMD (in one embodiment wet AMD, dry AMD, intermediate AMD, advanced AMD, and geographic atrophy (GA)), macular degeneration, macular edema, DME (in one embodiment focal, non-center DME and diffuse, center-involved DME), retinopathy, diabetic retinopathy (DR) (in one embodiment proliferative DR (PDR), non-proliferative DR (NPDR), and high-altitude DR.

15 In a further aspect, the invention provides for the use of an antibody that binds to human VEGF and human IL-1beta in the manufacture or preparation of a medicament. In one aspect, the medicament is for treatment of a vascular disease. In a further aspect, the medicament is for use in a method of treating a vascular disease comprising administering to an individual having a vascular disease an effective amount of the medicament. In one such aspect, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below.

25 In one aspect, the medicament is for treatment of an ocular disease. In a further aspect, the medicament is for use in a method of treating an ocular disease comprising administering to an individual having an ocular disease an effective amount of the medicament. In one such aspect, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below.

30 In a further aspect, the invention provides a method for treating a vascular disease. In one aspect, the method comprises administering to an individual having such vascular disease an effective amount of an antibody that binds to human VEGF and human IL-1beta. In one such aspect, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below.

In a further aspect, the invention provides a method for treating an ocular disease. In one aspect, the method comprises administering to an individual having such ocular disease an effective amount of an antibody that binds to human VEGF and human IL-1beta. In one such aspect, the method further comprises administering  
5 to the individual an effective amount of at least one additional therapeutic agent, as described below.

An “individual” according to any of the above aspects may be a human.

In a further aspect, the invention provides pharmaceutical compositions comprising any of the antibodies that bind to human VEGF and human IL-1beta provided herein, e.g., for use in any of the above therapeutic methods. In one aspect,  
10 a pharmaceutical composition comprises any of the antibodies that bind to human VEGF and human IL-1beta provided herein and a pharmaceutically acceptable carrier. In another aspect, a pharmaceutical composition comprises any of the antibodies that bind to human VEGF and human IL-1beta provided herein and at  
15 least one additional therapeutic agent, e.g., as described below.

Antibodies of the invention can be administered alone or used in a combination therapy. For instance, the combination therapy includes administering an antibody of the invention and administering at least one additional therapeutic agent (e.g. one, two, three, four, five, or six additional therapeutic agents).

For example, in certain embodiments, any of the preceding methods further comprises administering one or more additional compounds. In certain  
20 embodiments, the antibody that binds to human VEGF and human IL-1beta provided herein is administered simultaneously with the additional compound(s). In certain embodiments, the antibody that binds to human VEGF and human IL-1beta is  
25 administered before or after the additional compound(s). In certain embodiments, the additional compound binds to a second biological molecule selected from the group consisting of IL-6; IL-6R; IL-13; IL-13R; PDGF; angiopoietin; Ang2; Tie2; S1P; integrins  $\alpha v \beta 3$ ,  $\alpha v \beta 5$ , and  $\alpha 5 \beta 1$ ; betacellulin; apelin/APJ; erythropoietin; complement factor D; TNF $\alpha$ ; HtrA1; a VEGF receptor; ST-2 receptor; and proteins  
30 genetically linked to AMD risk, such as complement pathway components C2, factor B, factor H, CFHR3, C3b, C5, C5a, and C3a; HtrA1; ARMS2; TIMP3; HLA; interleukin-8 (IL-8); CX3CR1; TLR3; TLR4; CETP; LPC; COL10A1; and TNFRSF10A. In certain embodiments, the additional compound is an antibody or antigen-binding fragment thereof.

In certain embodiments according to (or as applied to) any of the embodiments above, the ocular disorder is an intraocular neovascular disease selected from the group consisting of proliferative retinopathies, choroidal neovascularization (CNV), age-related macular degeneration (AMD), diabetic and other ischemia-related retinopathies, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, retinal vein occlusion (RVO), including CRVO and BRVO, corneal neovascularization, retinal neovascularization, and retinopathy of prematurity (ROP).

In some instances, an antibody that binds to human VEGF and human IL-1beta provided herein may be administered in combination with at least one additional therapeutic agent for treatment of an ocular disorder, for example, an ocular disorder described herein (e.g., AMD (e.g., wet AMD), DME, DR, RVO, or GA). Exemplary additional therapeutic agents for combination therapy for treatment of ocular disorders include, without limitation, anti-angiogenic agents, such as VEGF antagonists, including, for example, anti-VEGF antibodies (e.g., the anti-VEGF Fab LUCENTIS® (ranibizumab)), soluble receptor fusion proteins (e.g., the recombinant soluble receptor fusion protein EYLEA® (aflibercept, also known as VEGF Trap Eye; Regeneron/Aventis)), aptamers (e.g., the anti-VEGF pegylated aptamer MACUGEN® (pegaptanib sodium; NeXstar Pharmaceuticals/OSI Pharmaceuticals)), and VEGFR tyrosine kinase inhibitors (e.g., 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474), 4-(4-fluoro-2-methylindol-5-ylloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171), vatalanib (PTK787), semaxaminib (SU5416; SUGEN), and SUTENT® (sunitinib)); Tryptophanyl-tRNA synthetase (TrpRS); squalamine; RETAANE® (anecortave acetate for depot suspension; Alcon, Inc.); Combretastatin A4 Prodrug (CA4P); MIFEPREX® (mifepristone-ru486); subtenon triamcinolone acetonide; intravitreal crystalline triamcinolone acetonide; matrix metalloproteinase inhibitors (e.g., Prinomastat (AG3340; Pfizer)); fluocinolone acetonide (including fluocinolone intraocular implant; Bausch & Lomb/Control Delivery Systems); linomide; inhibitors of integrin  $\beta 3$  function; angiostatin, and combinations thereof. These and other therapeutic agents that can be administered in combination with an antibody that binds to human VEGF and human IL-1beta of the invention are described, for example, in U.S. Patent Application No. US 2014/0017244, which is incorporated herein by reference in its entirety.

Further examples of additional therapeutic agents that can be used in combination with an antibody that binds to human VEGF and human IL-1betaas

provided herein for treatment of an ocular disorder (e.g., AMD, DME, DR, RVO, or GA), include, but are not limited to, VISUDYNE® (verteporfin; a light-activated drug that is typically used in conjunction with photodynamic therapy with a non-thermal laser), PKC412, Endovion (NS 3728; NeuroSearch A/S), neurotrophic factors (e.g., glial derived neurotrophic factor (GDNF) and ciliary neurotrophic factor (CNTF)), diltiazem, dorzolamide, PHOTOTROP®, 9-cis-retinal, eye medication (e.g., phospholine iodide, echthiophate, or carbonic anhydrase inhibitors), veovastat (AE-941; AEterna Laboratories, Inc.), Sima-027 (AGF-745; Sima Therapeutics, Inc.), neurotrophins (including, by way of example only, NT-4/5, Genentech), Cand5 (Acuity Pharmaceuticals), INS-37217 (Inspire Pharmaceuticals), integrin antagonists (including those from Jerini AG and Abbott Laboratories), EG-3306 (Ark Therapeutics Ltd.), BDM-E (BioDiem Ltd.), thalidomide (as used, for example, by EntreMed, Inc.), cardiotrophin-1 (Genentech), 2-methoxyestradiol (Allergan/Oculex), DL-8234 (Toray Industries), NTC-200 (Neurotech), tetrathiomolybdate (University of Michigan), LYN-002 (Lynkeus Biotech), microalgal compound (Aquasearch/Albany, Mera Pharmaceuticals), D-9120 (Celltech Group plc), ATX-S10 (Hamamatsu Photonics), TGF-beta 2 (Genzyme/Celtrix), tyrosine kinase inhibitors (e.g., those from Allergan, SUGEN, or Pfizer), NX-278-L (NeXstar Pharmaceuticals/Gilead Sciences), Opt-24 (OPTIS France SA), retinal cell ganglion neuroprotectants (Cogent Neurosciences), N-nitropyrazole derivatives (Texas A&M University System), KP-102 (Krenitsky Pharmaceuticals), cyclosporin A, therapeutic agents used in photodynamic therapy (e.g., VISUDYNE®; receptor-targeted PDT, Bristol-Myers Squibb, Co.; porfimer sodium for injection with PDT; verteporfin, QLT Inc.; rostoporfin with PDT, Miravent Medical Technologies; talaporfin sodium with PDT, Nippon Petroleum; and motexafin lutetium, Pharmacyclics, Inc.), antisense oligonucleotides (including, by way of example, products tested by Novagali Pharma SA and ISIS-13650, Ionis Pharmaceuticals), and combinations thereof.

An antibody that binds to human VEGF and human IL-1beta as provided herein may be administered in combination with a therapy or surgical procedure for treatment of an ocular disorder (e.g., AMD, DME, DR, RVO, or GA), including, for example, laser photocoagulation (e.g., panretinal photocoagulation (PRP)), drusen lasering, macular hole surgery, macular translocation surgery, implantable miniature telescopes, PHI-motion angiography (also known as micro-laser therapy and feeder vessel treatment), proton beam therapy, microstimulation therapy, retinal detachment and vitreous surgery, scleral buckle, submacular surgery, transpupillary thermotherapy, photosystem I therapy, use of RNA interference (RNAi),

extracorporeal rheopheresis (also known as membrane differential filtration and rheotherapy), microchip implantation, stem cell therapy, gene replacement therapy, ribozyme gene therapy (including gene therapy for hypoxia response element, Oxford Biomedica; Lentipak, Genetix; and PDEF gene therapy, GenVec),  
5 photoreceptor/retinal cells transplantation (including transplantable retinal epithelial cells, Diacrin, Inc.; retinal cell transplant, e.g., Astellas Pharma US, Inc., ReNeuron, CHA Biotech), acupuncture, and combinations thereof.

In some instances, an antibody that binds to human VEGF and human IL-1beta can be administered in combination with an anti-angiogenic agent for treatment of  
10 an ocular disorder (e.g., AMD, DME, DR, RVO, or GA). Any suitable anti-angiogenic agent can be used in combination with an antibody that binds to human VEGF and human IL-1beta of the invention, including, but not limited to, those listed by Carmeliet et al. *Nature* 407:249-257, 2000. In some embodiments, the anti-angiogenic agent is a VEGF antagonist, including, but not limited to, an anti-VEGF  
15 antibody (e.g., the anti-VEGF Fab LUCENTIS® (ranibizumab), RTH-258 (formerly ESBA-1008, an anti-VEGF single-chain antibody fragment; Novartis), or a bispecific anti-VEGF antibody (e.g., an anti-VEGF/anti-angiopoietin 2 bispecific antibody such as faricimab; Roche)), a soluble recombinant receptor fusion protein (e.g., EYLEA® (aflibercept)), a VEGF variant, a soluble VEGFR fragment, an  
20 aptamer capable of blocking VEGF (e.g., pegaptanib) or VEGFR, a neutralizing anti-VEGFR antibody, a small molecule inhibitor of VEGFR tyrosine kinases, an anti-VEGF DARPIn® (e.g., abicipar pegol, Molecular Partners AG/Allergan), a small interfering RNAs which inhibits expression of VEGF or VEGFR, a VEGFR tyrosine  
25 kinase inhibitor (e.g., 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474), 4-(4-fluoro-2-methylindol-5-ylloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171), vatalanib (PTK787), semaxaminib (SU5416; SUGEN), and SUTENT® (sunitinib)), and combinations thereof.

Other suitable anti-angiogenic agents that may be administered in combination  
30 with an antibody that binds to human VEGF and human IL-1beta as provided herein for treatment of an ocular disorder (e.g., AMD, DME, DR, RVO, or GA) include corticosteroids, angiostatic steroids, anecortave acetate, angiostatin, endostatin, tyrosine kinase inhibitors, matrix metalloproteinase (MMP) inhibitors, insulin-like growth factor-binding protein 3 (IGFBP3), stromal derived factor (SDF-1)  
35 antagonists (e.g., anti-SDF-1 antibodies), pigment epithelium-derived factor (PEDF), gamma-secretase, Delta-like ligand 4, integrin antagonists, hypoxia-

inducible factor (HIF)-1 $\alpha$  antagonists, protein kinase CK2 antagonists, agents that inhibit stem cell (e.g., endothelial progenitor cell) homing to the site of neovascularization (e.g., an anti-vascular endothelial cadherin (CD-144) antibody and/or an anti-SDF-1 antibody), and combinations thereof.

5           In a further example, in some instances, an antibody that binds to human VEGF and human IL-1 $\beta$ , and/or polymeric formulation thereof, can be administered in combination with an agent that has activity against neovascularization for treatment of an ocular disorder (e.g., AMD, DME, DR, RVO, or GA), such as an anti-inflammatory drug, a mammalian target of rapamycin (mTOR) inhibitor (e.g.,  
10   rapamycin, AFINITOR® (everolimus), and TORISEL® (temsirolimus)), cyclosporine, a tumor necrosis factor (TNF) antagonist (e.g., an anti-TNF $\alpha$  antibody or antigen-binding fragment thereof (e.g., infliximab, adalimumab, certolizumab pegol, and golimumab) or a soluble receptor fusion protein (e.g., etanercept)), an anti-complement agent, a nonsteroidal antiinflammatory agent (NSAID), or  
15   combinations thereof.

          In a still further example, in some instances, an antibody that binds to human VEGF and human IL-1 $\beta$  can be administered in combination with an agent that is neuroprotective and can potentially reduce the progression of dry AMD to wet AMD, such as the class of drugs called the “neurosteroids,” which include drugs such as  
20   dehydroepiandrosterone (DHEA) (brand names: PRASTERA™ and FIDELIN®), dehydroepiandrosterone sulfate, and pregnenolone sulfate.

          Any suitable AMD therapeutic agent can be administered as an additional therapeutic agent in combination with an antibody that binds to human VEGF and human IL-1 $\beta$  as provided herein for treatment of an ocular disorder (e.g., AMD,  
25   DME, DR, RVO, or GA), including, but not limited to, a VEGF antagonist, for example, an anti-VEGF antibody (e.g., LUCENTIS® (ranibizumab), RTH-258 (formerly ESBA-1008, an anti-VEGF single-chain antibody fragment; Novartis), or a bispecific anti-VEGF antibody (e.g., an anti-VEGF/anti-angiopoietin 2 bispecific antibody such as faricimab; Roche)), a soluble VEGF receptor fusion protein (e.g.,  
30   EYLEA® (aflibercept)), an anti-VEGF DARPIn® (e.g., abicipar pegol; Molecular Partners AG/Allergan), or an anti-VEGF aptamer (e.g., MACUGEN® (pegaptanib sodium)); a platelet-derived growth factor (PDGF) antagonist, for example, an anti-PDGF antibody, an anti-PDGFR antibody (e.g., REGN2176-3), an anti-PDGF-BB pegylated aptamer (e.g., FOVISTA®; Ophthotech/Novartis), a soluble PDGFR  
35   receptor fusion protein, or a dual PDGF/VEGF antagonist (e.g., a small molecule

inhibitor (e.g., DE-120 (Santen) or X-82 (TyrogeneX)) or a bispecific anti-PDGF/anti-VEGF antibody)); VISUDYNE® (verteporfin) in combination with photodynamic therapy; an antioxidant; a complement system antagonist, for example, a complement factor C5 antagonist (e.g., a small molecule inhibitor (e.g., ARC-1905; Ophotech) or an anti-C5 antibody (e.g., LFG-316; Novartis), a properdin antagonist (e.g., an anti-properdin antibody, e.g., CLG-561; Alcon), or a complement factor D antagonist (e.g., an anti-complement factor D antibody, e.g., lampalizumab; Roche)); a C3 blocking peptide (e.g., APL-2, Appellis); a visual cycle modifier (e.g., emixustat hydrochloride); squalamine (e.g., OHR-102; Ohr Pharmaceutical); vitamin and mineral supplements (e.g., those described in the Age-Related Eye Disease Study 1 (AREDS1; zinc and/or antioxidants) and Study 2 (AREDS2; zinc, antioxidants, lutein, zeaxanthin, and/or omega-3 fatty acids)); a cell-based therapy, for example, NT-501 (Renexus); PH-05206388 (Pfizer), huCNS-SC cell transplantation (StemCells), CNTO-2476 (umbilical cord stem cell line; Janssen), OpRegen (suspension of RPE cells; Cell Cure Neurosciences), or MA09-hRPE cell transplantation (Ocata Therapeutics); a tissue factor antagonist (e.g., hIcon1; Iconic Therapeutics); an alpha-adrenergic receptor agonist (e.g., brimonidine tartrate; Allergan); a peptide vaccine (e.g., S-646240; Shionogi); an amyloid beta antagonist (e.g., an anti-beta amyloid monoclonal antibody, e.g., GSK-933776); an S1P antagonist (e.g., an anti-S1P antibody, e.g., iSONEP™; Lpath Inc); a ROBO4 antagonist (e.g., an anti-ROBO4 antibody, e.g., DS-7080a; Daiichi Sankyo); a lentiviral vector expressing endostatin and angiostatin (e.g., RetinoStat); and any combination thereof. In some instances, AMD therapeutic agents (including any of the preceding AMD therapeutic agents) can be co-formulated. For example, the anti-PDGFR antibody REGN2176-3 can be co-formulated with aflibercept (EYLEA®). In some instances, such a co-formulation can be administered in combination with an antibody that binds to human VEGF and human IL-1beta of the invention. In some instances, the ocular disorder is AMD (e.g., wet AMD).

An antibody that binds to human VEGF and human IL-1beta of the invention can be administered in combination with LUCENTIS® (ranibizumab) for treatment of an ocular disorder (e.g., AMD, DME, DR, RVO, or GA). In some instances, the ocular disorder is AMD (e.g., wet AMD). In some instances, the ocular disorder is GA.

An antibody that binds to human VEGF and human IL-1beta of the invention can be administered in combination with EYLEA® (aflibercept) for treatment of an

ocular disorder (e.g., AMD, DME, DR, RVO, or GA). In some instances, the ocular disorder is AMD (e.g., wet AMD). In some instances, the ocular disorder is GA.

5 An antibody that binds to human VEGF and human IL-1beta of the invention can be administered in combination with MACUGEN® (pegaptanib sodium) for treatment of an ocular disorder (e.g., AMD, DME, DR, RVO, or GA). In some instances, the ocular disorder is AMD (e.g., wet AMD). In some instances, the ocular disorder is GA.

10 An antibody that binds to human VEGF and human IL-1beta of the invention can be administered in combination with VISUDYNE® (verteporfin) in combination with photodynamic therapy for treatment of an ocular disorder (e.g., AMD, DME, DR, RVO, or GA). In some instances, the ocular disorder is AMD (e.g., wet AMD). In some instances, the ocular disorder is GA.

15 An antibody that binds to human VEGF and human IL-1beta of the invention can be administered in combination with a PDGF antagonist for treatment of an ocular disorder (e.g., AMD, DME, DR, RVO, or GA). Exemplary PDGF antagonists which may be used in combination with an antibody that binds to human VEGF and human IL-1beta of the invention include an anti-PDGF antibody, an anti-PDGFR antibody, a small molecule inhibitor (e.g., squalamine), an anti-PDGF-B pegylated aptamer such as FOVISTA® (E10030; Ophthotech/Novartis), or a dual  
20 PDGF/VEGF antagonist (e.g., a small molecule inhibitor (e.g., DE-120 (Santen) or X-82 (TyrogeneX)) or a bispecific anti-PDGF/anti-VEGF antibody). For example, FOVISTA® can be administered as an adjunct therapy to an antibody that binds to human VEGF and human IL-1beta of the invention. OHR-102 can be administered in combination with VEGF antagonists such as LUCENTIS® or EYLEA®. In some  
25 embodiments, an antibody that binds to human VEGF and human IL-1beta of the invention can be administered in combination with OHR-102, LUCENTIS®, and/or EYLEA®. In some instances, the ocular disorder is AMD (e.g., wet AMD). In some instances, the ocular disorder is GA.

30 An antibody that binds to human VEGF and human IL-1beta of the invention can be administered in combination with RTH-258 for treatment of an ocular disorder (e.g., AMD, DME, DR, RVO, or GA). RTH-258 can be administered, for example, by intravitreal injection or eye infusion. In some instances, the ocular disorder is AMD (e.g., wet AMD). In some instances, the ocular disorder is GA.

An antibody that binds to human VEGF and human IL-1beta of the invention can be administered in combination with abicipar pegol for treatment of an ocular disorder (e.g., AMD, DME, DR, RVO, or GA). In some instances, the ocular disorder is AMD (e.g., wet AMD). In some instances, the ocular disorder is GA.

5 Any suitable DME and/or DR therapeutic agent can be administered in combination with an antibody that binds to human VEGF and human IL-1beta of the invention for treatment of an ocular disorder (e.g., AMD, DME, DR, RVO, or GA), including, but not limited, to a VEGF antagonist (e.g., LUCENTIS® or EYLEA®), a corticosteroid (e.g., a corticosteroid implant (e.g., OZURDEX® (dexamethasone  
10 intravitreal implant) or ILUVIEN® (fluocinolone acetonide intravitreal implant)) or a corticosteroid formulated for administration by intravitreal injection (e.g., triamcinolone acetonide)), or combinations thereof. In some instances, the ocular disorder is DME and/or DR.

15 An antibody that binds to human VEGF and human IL-1beta of the invention can be administered in combination with LUCENTIS® (ranibizumab) for treatment of DME and/or DR (e.g., NPDR or PDR).

An antibody that binds to human VEGF and human IL-1beta of the invention can be administered in combination with EYLEA® (aflibercept) for treatment of DME and/or DR (e.g., NPDR or PDR).

20 An antibody that binds to human VEGF and human IL-1beta of the invention can be administered in combination with OZURDEX® (dexamethasone intravitreal implant) for treatment of DME and/or DR.

25 An antibody that binds to human VEGF and human IL-1beta of the invention can be administered in combination with ILUVIEN® (dexamethasone intravitreal implant) for treatment of DME and/or DR.

30 In some cases, the TAO/PRN treatment regimen or TAE treatment regimen may be used to administer an AMD therapeutic agent (e.g., ranibizumab or aflibercept) in combination with an antibody that binds to human VEGF and human IL-1beta of the invention, and/or polymeric formulation thereof. In some instances, the ocular disorder is AMD (e.g., wet AMD). In some instances, the ocular disorder is GA.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate

formulations), and separate administration, in which case, administration of the antibody that binds to human VEGF and human IL-1beta of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one embodiment, administration of the antibody that binds to human VEGF and human IL-1beta of the invention and administration of an additional therapeutic agent occur within about one, two, three, four, or five months, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.

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

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

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's

clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1  $\mu\text{g/kg}$  to 15  $\text{mg/kg}$  (e.g., 0.1 $\text{mg/kg}$ -10 $\text{mg/kg}$ ) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1  $\mu\text{g/kg}$  to 100  $\text{mg/kg}$  or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05  $\text{mg/kg}$  to about 10  $\text{mg/kg}$ . Thus, one or more doses of about 0.5  $\text{mg/kg}$ , 2.0  $\text{mg/kg}$ , 4.0  $\text{mg/kg}$  or 10  $\text{mg/kg}$  (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g., every week or every three weeks (e.g., such that the patient receives from about two to about twenty, or, e.g., about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. The progress of this therapy is easily monitored by conventional techniques and assays.

#### **E. Articles of Manufacture**

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this aspect of the invention may further comprise a package insert indicating that the compositions can be used to treat a

particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

### 3. Specific embodiments of the invention

In the following specific embodiments of the invention are listed.

1. An antibody that binds to human VEGF and to human IL-1beta, comprising a VEGF paratope and an IL-1beta paratope within one cognate pair of a variable light chain domain (VL domain) and a variable heavy chain domain (VH domain), wherein the VEGF paratope comprises amino acid residues from CDR-H2, CDR-L1 and CDR-L3 of the antibody, wherein the IL-1beta paratope comprises amino acid residues from the CDR-H1, CDR-H3 and CDR-L2 of the antibody.
2. An antibody that binds to human VEGF and to human IL-1beta, comprising a VEGF paratope and an IL-1beta paratope within one cognate pair of a variable light chain domain (VL domain) and a variable heavy chain domain (VH domain), wherein the pair of the variable light chain domain and the variable heavy chain domain simultaneously binds to human VEGF and human IL-1beta.
3. An antibody that binds to human VEGF and to human IL-1beta, comprising a VEGF paratope and an IL-1beta paratope within one cognate pair of a variable light chain domain (VL domain) and a variable heavy chain domain (VH domain), wherein none of the amino acids that are comprised in the VEGF paratope are comprised in the IL-1beta paratope.
4. An antibody that binds to human VEGF and to human IL-1beta, comprising a VEGF paratope and an IL-1beta paratope within one cognate pair of a variable light chain domain (VL domain) and a variable heavy chain domain (VH domain), wherein the antibody binds to the same epitope on human VEGF and to the same epitope on human IL-1beta as an antibody with a variable heavy chain domain of SEQ ID NO: 11 and a variable light chain domain of SEQ ID NO: 12.

5. An antibody that binds to human VEGF and to human IL-1beta, comprising a VEGF paratope and an IL-1beta paratope within one cognate pair of a variable light chain domain (VL domain) and a variable heavy chain domain (VH domain), wherein
- the VEGF paratope comprises amino acid residues from CDR-H2, CDR-L1 and CDR-L3 of the antibody, wherein the IL-1beta paratope comprises amino acid residues from the CDR-H1, CDR-H3 and CDR-L2 of the antibody; and/or
  - the pair of the variable light chain domain and the variable heavy chain domain simultaneously binds to human VEGF and human IL-1beta; and/or
  - none of the amino acids that are comprised in the VEGF paratope are comprised in the IL-1beta paratope; and/or
  - the antibody binds to the same epitope on human VEGF and to the same epitope on human IL-1beta as an antibody with a variable heavy chain domain of SEQ ID NO: 11 and a variable light chain domain of SEQ ID NO: 12; and/or
  - an antibody Fab fragment of the antibody binds (i) to human VEGF121 with a  $K_D$  of less than 10 pM as measured by surface plasmon resonance, and (ii) to human IL-1beta with a  $K_D$  of less than 30 pM as measured by surface plasmon resonance; and/or
  - an antibody Fab fragment of the antibody exhibits an aggregation onset temperature of more than 70 °C; and/or
  - an antibody Fab fragment of the antibody exhibits a melting temperature of more than 80 °C as measured by dynamic light scattering; and/or
  - binding of an antibody Fab fragment of the antibody to human VEGF inhibits binding of VEGF to VEGFR2 with an  $IC_{50}$  of less than 50 nM as measured by surface plasmon resonance; and wherein binding of an antibody Fab fragment of the antibody to human IL-1beta inhibits binding of IL-1beta to IL-1betaR1 with an  $IC_{50}$  of less than 30 nM as measured by surface plasmon resonance.
6. The antibody of one of one of the preceding embodiments, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ

ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8.

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7. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8.
8. The antibody of one of one of the preceding embodiments, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.
9. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-

5 L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.

10 10. The antibody of one of the preceding embodiments, comprising a VH domain comprising amino acid residues E2, G26, V28, K30, W31, N35b, D35c, K52a, D55, H56, Y58, T61, K62, F63, I64, R66, R83, K94, D95, V96, F98, and D101, and a VL domain comprising amino acid residues I2, Y27, W27a, S27c, S27d, L32, Y49, D50, Y53, K54, L56, G57, E67, D68, Q69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.

15 11. The antibody of embodiment 10, comprising

- 20 - a VEGF paratope comprising the following amino acid residues in the VH domain D55, H56, Y58, T61, K62, F63, I64, R66, and R83, and the following amino acid residues in the VL domain I2, Y27, W27a, S27c, S27d, E67, D68, Q69, R92, Y93, H94, and Y96; and
- an IL-1beta paratope comprising the following amino acid residues in the VH domain E2, G26, V28, K30, W31, N35b, D35c, K52a, K94, D95, V96, F98, and D101, and the following amino acid residues in the VL domain L32, Y49, D50, Y53, K54, L56, G57, Y91.

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30 12. An antibody that specifically binds to human VEGF and to human IL-1beta, comprising within one pair of a VL domain and a VH domain: (i) a VH domain comprising amino acid residues E2, G26, V28, K30, W31, N35b, D35c, K52a, D55, H56, Y58, T61, K62, F63, I64, R66, R83, K94, D95, V96, F98, and D101, and (ii) a VL domain comprising amino acid residues I2, Y27, W27a, S27c, S27d, L32, Y49, D50, Y53, K54, L56, G57, E67, D68, Q69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.

35 13. The antibody of embodiment 12, comprising

- a VEGF paratope comprising the following amino acid residues in the VH domain: D55, H56, Y58, T61, K62, F63, I64, R66, and R83, and the

following amino acid residues in the VL domain: I2, Y27, W27a, S27c, S27d, E67, D68, Q69, R92, Y93, H94, and Y96; and

- an IL-1beta paratope comprising the following amino acid residues in the VH domain: E2, G26, V28, K30, W31, N35b, D35c, K52a, K94, D95, V96, F98, and D101, and the following amino acid residues in the VL domain: L32, Y49, D50, Y53, K54, L56, G57, Y91.

14. The antibody of any one of the preceding embodiments, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12.

15. The antibody of any one of the preceding embodiments, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11, wherein the VH domain comprises amino acid residues E2, G26, V28, K30, W31, N35b, D35c, K52a, D55, H56, Y58, T61, K62, F63, I64, R66, R83, K94, D95, V96, F98, and D101; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12, wherein the VL domain comprises amino acid residues I2, Y27, W27a, S27c, S27d, L32, Y49, D50, Y53, K54, L56, G57, E67, D68, Q69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.

16. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12.

17. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11, wherein the VH domain comprises amino acid residues E2, G26, V28, K30, R66, R83, and K94; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12, wherein the VL domain comprises amino acid residues I2, Y49, G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.
18. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12.

19. The antibody of any one of the preceding embodiments, comprising (a) a VH domain comprising an amino acid sequence of SEQ ID NO:11 with up to 15 amino acid substitutions; and (b) a variable light chain domain comprising an amino acid sequence of SEQ ID NO:12 with up to 15 amino acid substitutions.
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20. The antibody of any one of the preceding embodiments, comprising (a) a VH domain comprising an amino acid sequence of SEQ ID NO:11 with up to 15 amino acid substitutions, wherein the amino acid substitutions are located at positions 3 to 25, 36 to 49, 97 to 82c, 84 to 93, or 103 to 113 of SEQ ID NO:11; and (b) a variable light chain domain comprising an amino acid sequence of SEQ ID NO:12 with up to 15 amino acid substitutions, wherein the amino acid substitutions are located at positions 1, 4, 6, 8 to 23, 35 to 48, 58 to 66, 70 to 88, or 98 to 107 of SEQ ID NO:12, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.
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21. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence of SEQ ID NO:11 with up to 15 amino acid substitutions; and (b) a variable light chain domain comprising an amino acid sequence of SEQ ID NO:12 with up to 15 amino acid substitutions.
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22. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light
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- chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, and comprising (a) a VH domain comprising an amino acid sequence of SEQ ID NO:11 with up to 15 amino acid substitutions; and (b) a variable light chain domain comprising an amino acid sequence of SEQ ID NO:12 with up to 15 amino acid substitutions.
23. The antibody of any one of the preceding embodiments, comprising a VH sequence of SEQ ID NO:11 and a VL sequence of SEQ ID NO:12.
24. An antibody that specifically binds to human VEGF and to human IL-1beta, comprising a VH sequence of SEQ ID NO:11 and a VL sequence of SEQ ID NO:12.
25. The antibody of any one of the preceding embodiments, comprising a heavy chain amino acid sequence of SEQ ID NO:20 and a light chain amino acid sequence of SEQ ID NO:19.
26. An antibody that specifically binds to human VEGF and to human IL-1beta, comprising a heavy chain amino acid sequence of SEQ ID NO:20 and a light chain amino acid sequence of SEQ ID NO:19.
27. The antibody of any one of the preceding embodiments, comprising a heavy chain amino acid sequence of SEQ ID NO:18 and a light chain amino acid sequence of SEQ ID NO:19.
28. An antibody that specifically binds to human VEGF and to human IL-1beta, comprising a heavy chain amino acid sequence of SEQ ID NO:18 and a light chain amino acid sequence of SEQ ID NO:19.
29. The antibody of any one of the preceding embodiments, wherein an antibody Fab fragment of the antibody binds (i) to human VEGF121 with a  $K_D$  of less than 10 pM as measured by surface plasmon resonance, and (ii) to human IL-1beta with a  $K_D$  of less than 30 pM as measured by surface plasmon resonance.

30. An antibody that specifically binds to human VEGF and to human IL-1beta wherein an antibody Fab fragment of the antibody binds (i) to human VEGF121 with a  $K_D$  of less than 10 pM as measured by surface plasmon resonance, and (ii) to human IL-1beta with a  $K_D$  of less than 30 pM as measured by surface plasmon resonance.
31. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, wherein an antibody Fab fragment of the antibody binds (i) to human VEGF121 with a  $K_D$  of less than 10 pM as measured by surface plasmon resonance, and (ii) to human IL-1beta with a  $K_D$  of less than 30 pM as measured by surface plasmon resonance.
32. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, wherein an antibody Fab fragment of the antibody binds (i) to human VEGF121 with a  $K_D$  of less than 10 pM as measured by surface plasmon resonance, and (ii) to human IL-1beta with a  $K_D$  of less than 30 pM as measured by surface plasmon resonance.

33. An antibody that specifically binds to human VEGF and to human IL-1beta, comprising within one pair of a VH and VL domain: (i) a VH domain comprising amino acid residues E2, G26, V28, K30, W31, N35b, D35c, K52a, D55, H56, Y58, T61, K62, F63, I64, R66, R83, K94, D95, V96, F98, and D101, and (ii) VL domain comprising amino acid residues I2, Y27, W27a, S27c, S27d, L32, Y49, D50, Y53, K54, L56, G57, E67, D68, Q69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, wherein an antibody Fab fragment of the antibody binds (i) to human VEGF121 with a  $K_D$  of less than 10 pM as measured by surface plasmon resonance, and (ii) to human IL-1beta with a  $K_D$  of less than 30 pM as measured by surface plasmon resonance.
34. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12; wherein an antibody Fab fragment of the antibody binds (i) to human VEGF121 with a  $K_D$  of less than 10 pM as measured by surface plasmon resonance, and (ii) to human IL-1beta with a  $K_D$  of less than 30 pM as measured by surface plasmon resonance.
35. The antibody of any one of the preceding embodiments, wherein an antibody Fab fragment of the antibody exhibits an aggregation onset temperature of more than 70 °C.
36. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein an antibody Fab fragment of the antibody exhibits an aggregation onset temperature of more than 70 °C.

37. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, wherein an antibody Fab fragment of the antibody exhibits an aggregation onset temperature of more than 70 °C.
38. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, wherein an antibody Fab fragment of the antibody exhibits an aggregation onset temperature of more than 70 °C.
39. An antibody that specifically binds to human VEGF and to human IL-1beta, comprising within one pair of a VH and VL domain: (i) a VH domain comprising amino acid residues E2, G26, V28, K30, W31, N35b, D35c, K52a, D55, H56, Y58, T61, K62, F63, I64, R66, R83, K94, D95, V96, F98, and D101, and (ii) a VL domain comprising amino acid residues I2, Y27, W27a, S27c, S27d, L32, Y49, D50, Y53, K54, L56, G57, E67, D68, Q69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, wherein an antibody Fab fragment of the antibody exhibits an aggregation onset temperature of more than 70 °C.

40. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12; wherein an antibody Fab fragment of the antibody exhibits an aggregation onset temperature of more than 70 °C.
41. The antibody of any one of the preceding embodiments, wherein an antibody Fab fragment of the antibody exhibits a melting temperature of more than 80 °C as measured by dynamic light scattering.
42. An antibody that specifically binds to human VEGF and to human IL-1beta, , wherein an antibody Fab fragment of the antibody exhibits a melting temperature of more than 80 °C as measured by dynamic light scattering.
43. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, wherein an antibody Fab fragment of the antibody exhibits a melting temperature of more than 80 °C as measured by dynamic light scattering.
44. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i)

- FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, wherein an antibody Fab fragment of the antibody exhibits a melting temperature of more than 80 °C as measured by dynamic light scattering.
45. An antibody that specifically binds to human VEGF and to human IL-1beta, comprising within one pair of a v VH and VL domain: (i) a VH domain comprising amino acid residues E2, G26, V28, K30, W31, N35b, D35c, K52a, D55, H56, Y58, T61, K62, F63, I64, R66, R83, K94, D95, V96, F98, and D101, and (ii) a VL domain comprising amino acid residues I2, Y27, W27a, S27c, S27d, L32, Y49, D50, Y53, K54, L56, G57, E67, D68, Q69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, wherein an antibody Fab fragment of the antibody exhibits a melting temperature of more than 80 °C as measured by dynamic light scattering.
46. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a (VL domain) comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12; wherein an antibody Fab fragment of the antibody exhibits a melting temperature of more than 80 °C as measured by dynamic light scattering.

47. The antibody of any one of the preceding embodiments, wherein binding of an antibody Fab fragment of the antibody to human VEGF inhibits binding of VEGF to VEGFR2 with an IC<sub>50</sub> of less than 50 nM as measured by surface plasmon resonance; and wherein binding of an antibody Fab fragment of the antibody to human IL-1beta inhibits binding of IL-1beta to IL-1betaR1 with an IC<sub>50</sub> of less than 30 nM as measured by surface plasmon resonance.
48. An antibody that specifically binds to human VEGF and to human IL-1beta, , wherein binding of an antibody Fab fragment of the antibody to human VEGF inhibits binding of VEGF to VEGFR2 with an IC<sub>50</sub> of less than 50 nM as measured by surface plasmon resonance; and wherein binding of an antibody Fab fragment of the antibody to human IL-1beta inhibits binding of IL-1beta to IL-1betaR1 with an IC<sub>50</sub> of less than 30 nM as measured by surface plasmon resonance.
49. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and wherein binding of an antibody Fab fragment of the antibody to human IL-1beta inhibits binding of IL-1beta to IL-1betaR1 with an IC<sub>50</sub> of less than 30 nM as measured by surface plasmon resonance.
50. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66, R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, (g) CDR-

5 L3 comprising the amino acid sequence of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3 comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering of the VH and VL domains is according to the Kabat numbering system, and wherein binding of an antibody Fab fragment of the antibody to human IL-1beta inhibits binding of IL-1beta to IL-1betaR1 with an IC50 of less than 30 nM as measured by surface plasmon resonance.

10 51. An antibody that specifically binds to human VEGF and to human IL-1beta, comprising within one pair of a VH and VL domain: (i) a VH domain comprising amino acid residues E2, G26, V28, K30, W31, N35b, D35c, K52a, D55, H56, Y58, T61, K62, F63, I64, R66, R83, K94, D95, V96, F98, and D101, and (ii) a  
15 VL domain comprising amino acid residues I2, Y27, W27a, S27c, S27d, L32, Y49, D50, Y53, K54, L56, G57, E67, D68, Q69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the VH and VL domains is according to the Kabat numbering system; and wherein binding of an antibody Fab fragment of the antibody to human IL-1beta inhibits binding of IL-1beta to IL-1betaR1 with an IC50 of less than 30 nM as measured by surface plasmon resonance.

20 52. An antibody that specifically binds to human VEGF and to human IL-1beta, wherein the antibody comprises a VH domain comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (c) CDR-H3 comprising the  
25 amino acid sequence of SEQ ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12; and wherein binding of an antibody Fab fragment of the antibody to human IL-1beta inhibits binding of IL-1beta to IL-1betaR1 with an IC50 of less than 30 nM as measured by surface plasmon  
30 resonance.

35 53. The antibody of any one of the preceding embodiments, which is a monoclonal antibody.

54. The antibody of any one of the preceding embodiments, which is an antibody fragment that binds to human VEGF and to human IL-1beta.
55. The antibody of any one of the preceding embodiments, wherein the antibody is bispecific.
56. The antibody of any one of the preceding embodiments, wherein the antibody is a Fab fragment.
57. The antibody of any one of the preceding embodiments, wherein the antibody is a bispecific antibody fragment.
58. The antibody of any one of the preceding embodiments, wherein the antibody is a multispecific antibody.
59. An isolated nucleic acid encoding the antibody of any of embodiments 1 to 58.
60. A host cell comprising the nucleic acid of embodiment 59.
61. An expression vector comprising the nucleic acid of embodiment 61.
62. A method of producing an antibody that binds to human VEGF and to human IL-1beta comprising culturing the host cell of embodiment 60 so that the antibody is produced.
63. The method of embodiment 62, further comprising recovering the antibody from the host cell.
64. An antibody produced by the method of embodiment 62 or 63.
65. A pharmaceutical formulation comprising the antibody of any one of embodiments 1 to 58 and a pharmaceutically acceptable carrier.
66. The antibody of any one of embodiments 1 to 58 for use as a medicament.
67. The antibody of any one of embodiments 1 to 58 for use in the treatment of a vascular disease.

68. The antibody of any one of embodiments 1 to 58 for use in the treatment of an ocular vascular disease.
- 5 69. Use of the antibody of any one of embodiments 1 to 58 or the pharmaceutical composition of embodiment 65 in the manufacture of a medicament.
70. Use of the antibody of any one of embodiments 1 to 58 or the pharmaceutical composition of embodiment 65 in the manufacture of a medicament for  
10 inhibiting angiogenesis.
71. A method of treating an individual having a vascular disease comprising administering to the individual an effective amount of the antibody of one of  
15 embodiments 1 to 58 or the pharmaceutical composition of embodiment 65.
72. A method of treating an individual having an ocular vascular disease comprising administering to the individual an effective amount of the antibody of one of  
embodiments 1 to 58 or pharmaceutical composition of embodiment 65.
- 20 73. A method of inhibiting angiogenesis in an individual comprising administering to the individual an effective amount of the antibody of any of embodiments 1 to 58 or the pharmaceutical composition of any of embodiments 65 to inhibit angiogenesis.

#### DESCRIPTION OF THE AMINO ACID SEQUENCES

25

|                    |                                                                                                                                                              |
|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>SEQ ID NO:1</b> | VH domain of 1HVL2.3<br><br>EQLVESGGGLVKPGGSLRLSCAASGMVFSWNAMSWVRQAPGK<br>GLEWVGSISPKGDHKYLN TKFIGRFTISRDN SKNTLYLQMN S<br>LRAEDTAVYYCAKD IGF FDVWGQGTLVTVSS |
| <b>SEQ ID NO:2</b> | VL domain of 1HVL2.3<br><br>AIYMHQEPSSLSASVGDRVTITCHGSYWLSNYLAWYQQKPGK<br>APKLLIYDASYRIIGVPSRFSGSGSHEDYTLTISSLQPEDFA<br>TYYCQQYRYHPYTFGHG TKVEIKR            |

|                     |                                                                                                                                                                                                                                                                                                  |
|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>SEQ ID NO:3</b>  | H-CDR1 of 1HVL2.3<br>WNAMS                                                                                                                                                                                                                                                                       |
| <b>SEQ ID NO:4</b>  | H-CDR2 of 1HVL2.3<br>SISPKGDHKYLN TKFIG                                                                                                                                                                                                                                                          |
| <b>SEQ ID NO:5</b>  | H-CDR3 of 1HVL2.3<br>DIGFFDV                                                                                                                                                                                                                                                                     |
| <b>SEQ ID NO:6</b>  | L-CDR1 of 1HVL2.3<br>HGSYWLSNYLA                                                                                                                                                                                                                                                                 |
| <b>SEQ ID NO:7</b>  | L-CDR2 of 1HVL2.3<br>DASYRII                                                                                                                                                                                                                                                                     |
| <b>SEQ ID NO:8</b>  | L-CDR3 of 1HVL2.3, 1HVL12.85, 1HVL5.15 and RO7200394<br>QQYRYHPYT                                                                                                                                                                                                                                |
| <b>SEQ ID NO:9</b>  | heavy chain of 1HVL2.3 Fab fragment<br><br>EQLVESGGGLVKPGGSLRLSCAASGMVFSWNAMSWVRQAPGK<br>GLEWVGSISPKGDHKYLN TKFIGRFTISRDN SKNTLYLQMNS<br>LRAEDTAVYYCAKDIGFFDVWGQGTLVTVSSASTKGPSVFPL<br>APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF<br>PAVLQSSGLYSLSSVVTVPSSSLGTKTYICNVNHKPSNTKVD<br>KKVEPKSCDKTHT |
| <b>SEQ ID NO:10</b> | light chain chain of 1HVL2.3 Fab fragment<br><br>AIYMHQEPSSLSASVGDRVITITCHGSYWLSNYLAWYQQKPGK<br>APKLLIYDASYRIIGVPSRFSGSGSHEDYTLTISSLQPEDFA<br>TYYCQQYRYHPYTFGHGKVEIKRTVAAPSVFIFPPSDEQLK<br>SGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDS<br>KSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN<br>RGEC        |

|                     |                                                                                                                                                                                                                                                                                                  |
|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>SEQ ID NO:11</b> | VH domain of 1HVL12.85 and RO7200394<br><br>DEQLVESGGGLVKPGGSLRLSCAAEGMVFKNWDMMSWVRQAPG<br>KGLEWVGSISKKGDKHYLNTKFIGRFTISRDNEDTLYLQMN<br>SLRAEDTAVYYCAKDVGFEDIWGQGTLLVTVSS                                                                                                                        |
| <b>SEQ ID NO:12</b> | VL domain of 1HVL12.85 and RO7200394<br><br>AIYMHQEPSSLSASVGDRVTITCHGSYWLSSLVAWYQQKPGK<br>APKLLIYDAKYKHLGVPSRFSGSKEDQEFTLTISLQPEDFA<br>TYQCQQRYRHPYTFGHGKVEIK                                                                                                                                    |
| <b>SEQ ID NO:13</b> | H-CDR1 of 1HVL12.85, 1HVL5.15 and RO7200394<br><br>WNDMS                                                                                                                                                                                                                                         |
| <b>SEQ ID NO:14</b> | H-CDR2 of 1HVL12.85, 1HVL5.15 and RO7200394<br><br>SISKKGDKHYLNTKFIG                                                                                                                                                                                                                             |
| <b>SEQ ID NO:15</b> | H-CDR3 of 1HVL12.85, 1HVL5.15 and RO7200394<br><br>DVGFFDI                                                                                                                                                                                                                                       |
| <b>SEQ ID NO:16</b> | L-CDR1 of 1HVL12.85 and RO7200394<br><br>HGSYWLSSLVA                                                                                                                                                                                                                                             |
| <b>SEQ ID NO:17</b> | L-CDR2 of 1HVL12.85, 1HVL5.15 and RO7200394<br><br>DAKYKHL                                                                                                                                                                                                                                       |
| <b>SEQ ID NO:18</b> | heavy chain of 1HVL12.85 Fab fragment<br><br>DEQLVESGGGLVKPGGSLRLSCAAEGMVFKNWDMMSWVRQAPG<br>KGLEWVGSISKKGDKHYLNTKFIGRFTISRDNEDTLYLQMN<br>SLRAEDTAVYYCAKDVGFEDIWGQGTLLVTVSSASTKGPSVFP<br>LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT<br>FPAVLQSSGLYSLSSVTVPSSSLGKTYICNVNHKPSNTKV<br>DKKVEPKSCDKTHT |

|                     |                                                                                                                                                                                                                                                                                                                                         |
|---------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>SEQ ID NO:19</b> | light chain chain of 1HVL12.85 and RO7200394 Fab fragment<br><br>AIYMHQEPSSLSASVGDRVITITCHGSYWLSSSLVAWYQQKPGK<br>APKLLIYDAKYKHLGVPSRFSGSKEDQEFTLTISLQPEDFA<br>TYYCQQYRYHPYTFGHGKVEIKRTVAAPSVFIFPPSDEQLK<br>SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS<br>KDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN<br>RGEN                              |
| <b>SEQ ID NO:20</b> | heavy chain of RO7200394 Fab fragment (SEQ ID NO: 19 with K196Q mutation)<br><br>DEQLVESGGGLVKKPGGSLRLSCAAEGMVFKNWDMSWVRQAPG<br>KGLEWVGSISKKGDKHYLNTKFIGRFTISRDNEDTLYLQMN<br>SLRAEDTAVYYCAKDVGFDFDIWGQGTLLVTVSSASTKGPSVFP<br>LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT<br>FPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKV<br>DKKVEPKSCDKTHT |
| <b>SEQ ID NO:21</b> | VH domain of 1HVL5.15<br><br>DETLVESGGGLVKKPGGSLRLSCAAEGMVFKNWDMSWVRQAPG<br>KGLEWVGSISKKGDKHYLNTKFIGRFTISRDNEDTLYLQMN<br>SLRAEDTAVYYCAKDVGFDFDIWGQGTLLVTVSS                                                                                                                                                                             |
| <b>SEQ ID NO:22</b> | VL domain of 1HVL5.15<br><br>AIYMHQEPSSLSASVGDRVITITCHGSYWLSSSLMAWYQQKPGK<br>APKLLIYDAKYKHLGVPSRFSGSGSHEDYTLTISLQPEDFA<br>TYYCQQYRYHPYTFGHGKVEIK                                                                                                                                                                                        |
| <b>SEQ ID NO:23</b> | L-CDR1 of 1HVL5.15<br><br>HGSYWLSSLMA                                                                                                                                                                                                                                                                                                   |
| <b>SEQ ID NO:24</b> | heavy chain of 1HVL5.15 Fab fragment<br><br>DETLVESGGGLVKKPGGSLRLSCAAEGMVFKNWDMSWVRQAPG<br>KGLEWVGSISKKGDKHYLNTKFIGRFTISRDNEDTLYLQMN<br>SLRAEDTAVYYCAKDVGFDFDIWGQGTLLVTVSSASTKGPSVFP<br>LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT<br>FPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKV<br>DKKVEPKSCDKTHT                                      |

|                     |                                                                                                                                                                                                                                                                                            |
|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>SEQ ID NO:25</b> | light chain chain of 1HVL5.15 Fab fragment<br><br>AIYMHQEPSSLSASVGDRVTTITCHGSYWLSSLMAWYQQKPGK<br>APKLLIYDAKYKHLGVPSRFSGSGSHEDYTLTISSLQPEDFA<br>TYYCQQYRYHPYTFGHGKVEIKRTVAAPSVFIFPPSDEQLK<br>SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS<br>KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFN<br>RGEN |
| <b>SEQ ID NO:26</b> | human VEGF<br><br>MNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVK<br>FMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRG<br>GCCNDEGLECVPTESNITMQIMRIKPHQGQHIGEMSFLQHN<br>KCECRPKKDRARQEKKSVRGKGKGQKRKRKKSRYKSWSVYVG<br>ARCCCLMPWSLPGPHPCGPCSERRKHLFVQDPQTCCKSCKNTD<br>SRCKARQLELNERTCRCDKPRR              |
| <b>SEQ ID NO:27</b> | human IL1beta<br><br>MAEVP ELASEMMAYYS GNEDDLFFEADGPKQMKCSFQDL DLC<br>PLDGGIQLRISDHHYSKGF RQAASVVVAMD KLRKMLVPCPQT<br>FQENDLSTFFPFI FEEEP IFFDTWDNE                                                                                                                                        |

## EXAMPLES

The following examples 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.

### **Example 1:**

#### **Generation of bispecific anti-VEGF/anti-IL-1beta Fab fragment**

A bispecific anti-VEGF/anti-IL-1beta Fab fragment was generated by independent screening of monospecific antibodies that bind to VEGF and IL-1beta with non-overlapping paratopes and subsequent merging of the amino acid sequence into a biparatopic VH/VL pair that binds to VEGF and IL-1beta, by a method as described before, e.g. in WO2012/163520.

Two distinct phage display libraries of synthetic Fab fragments were utilized, wherein in the first phage display library residues within the CDR-H1, CDR-H3 and CDR-L2 regions of the Fab fragments were diversified, and wherein in the second phage display library residues within the CDR-L1, CDR-L3 and CDR-H2 regions of the Fab fragments were diversified. In each library the other three CDR regions were kept non-diversified as invariant dummy sequence. In both libraries the CH1 domain of the Fab fragments was fused via a linker to a truncated gene-III protein to facilitate phage display.

The first library was enriched for binders against human IL-1beta, and the second library was enriched for binders against human VEGF-A, by phage library panning. Following panning, plasmid minipreps were generated for both enriched pools of phagemid vectors. The minipreps were digested with a restriction enzyme to excise the region encoding the truncated gene-III protein and re-circularized by ligation to obtain pools of expression vectors encoding soluble Fab fragments that were enriched for IL-1beta binders or for VEGF-A binders, respectively. These vector pools were transformed into TG1 E.coli cells and individual colonies were picked and cultured for soluble expression of individual Fab clones in microtiter plates. The supernatants comprising soluble Fab fragments were screened for binding to IL-1beta or VEGF-A using standard ELISA methods, and TG1 clones producing specific binders were subjected to DNA plasmid preparation and sequencing, to obtain pairs of VH and VL sequences specifically binding either to IL-1beta or to VEGF-A, respectively.

A pair of bispecific anti-VEGF/anti-IL-1beta VH and VL sequences was designed in silico by (1) replacing the irrelevant VH residues 52b to 65 in the VH sequence of an IL-1beta-specific Fab with selected VH residues 52b to 65 of a VEGF-A-specific Fab, thus substituting CDR-H2 residues potentially being part of the VEGF-A-specific paratope into the IL-1beta binder heavy chain, and (2) replacing the irrelevant VL residues 49 to 57 in the VL sequence of a VEGF-A-specific Fab with selected VL residues 49 to 57 of an IL-1beta-specific Fab, thus substituting CDR-L2 residues potentially being part of the IL-1beta-specific paratope into the VEGF-A binder light chain.

### **Example 2:**

#### **Expression of bispecific anti-VEGF/anti-IL-1beta Fab fragment 1HVL2.3**

The resulting designed pair of bispecific anti-VEGF/anti-IL-1beta VH and VL sequences was synthesized and cloned into an E.coli expression vector in frame

with gene sequences encoding CH1 and Ckappa domains. The vector was transformed into TG1 E.coli cells, and an individual colony was cultured for soluble expression of the bispecific antibody Fab fragment. The bispecific antibody was purified from the TG1 culture supernatant by affinity chromatography, and specific binding to both IL-1beta and VEGF-A was verified.

Bispecific anti-VEGF/anti-IL-1beta antibody "1HVL2.3" was selected, and is characterized by a heavy chain of SEQ ID NO:9 and a light chain of SEQ ID NO:10.

For further analyses anti-VEGF/anti-IL-1beta antibodies of the invention were transformed into and expressed from HEK293 cells by standard recombinant methods.

### **Example 3:**

#### **Characterization of bispecific anti-VEGF/anti-IL-1beta Fab fragment 1HVL2.3**

Binding affinity, hydrophilicity and thermal stability of bispecific antibody 1HVL2.3 were assessed as follows:

#### **VEGF Binding Kinetics as assessed by surface plasmon resonance (SPR):**

An anti-His capturing antibody (GE Healthcare 28995056) was immobilized to a Series S Sensor Chip C1 (GE Healthcare 29104990) using standard amine coupling chemistry resulting in a surface density of approximately 500 resonance units (RU). As running and dilution buffer, HBS-P+ (10 mM HEPES, 150 mM NaCl pH 7.4, 0.05% Surfactant P20) was used. Human VEGF121-His was captured to the surface with resulting ligand densities of approximately 10 and 20 RU, respectively. A dilution series of the bispecific anti-VEGF/anti-IL-1beta Fab fragment (1.2 – 100 nM, 1:3 dilution) was successively injected for 90s each, dissociation was monitored for 3600s at a flow rate of 30 µl/min (single cycle kinetics). The surface was regenerated by injecting 10 mM Glycine pH 1.5 for 60s. Bulk refractive index differences were corrected by subtracting blank injections and by subtracting the response obtained from the control flow cell without captured human VEGF121. Curve fitting was performed using the 1:1 Langmuir binding model within the Biacore evaluation software. To provide more robust fitting, the Multiple Rmax option was chosen for global fitting using both ligand densities.

**IL-1b Binding Kinetics as assessed by surface plasmon resonance (SPR):**

An anti-Fab capturing antibody (GE Healthcare 28958325) was immobilized to a Series S Sensor Chip C1 (GE Healthcare 29104990) using standard amine coupling chemistry resulting in a surface density of approximately 500 resonance units (RU). The bispecific anti-VEGF/anti-IL-1beta Fab fragment was captured to the surface with resulting capture levels of approximately 20 RU. A dilution series ranging from 0.74 to 60 nM (1:3 dilution) of either human IL-1 beta (PeproTech 200-01B) or cynomolgus IL-1 beta (Sino Biological 90010-CNAE) was injected for 90s, dissociation was monitored for at least 600s at a flow rate of 30 µl/min. The surface was regenerated by two consecutive injections of 10 mM Glycine pH 2.1 for 60s each. Bulk refractive index differences were corrected by subtracting blank injections and by subtracting the response obtained from the control flow cell without captured bispecific anti-VEGF/anti-IL-1beta Fab fragment. Curve fitting was performed using the 1:1 Langmuir binding model within the Biacore evaluation software.

**Hydrophobic interaction chromatography (HIC):**

Apparent hydrophobicity was determined by injecting 20 µg of the bispecific anti-VEGF/anti-IL-1beta Fab fragment onto a HIC-Ether-5PW (Tosoh) column equilibrated with 25 mM Na-phosphate, 1.5 M ammonium sulfate, pH 7.0. Elution was performed with a linear gradient from 0 to 100% buffer B (25 mM Na-phosphate, pH 7.0) within 60 minutes. Retention times were compared to protein standards with known hydrophobicity.

**Thermal stability:**

Samples of the bispecific anti-VEGF/anti-IL-1beta Fab fragment were prepared at a concentration of 1 mg/mL in 20 mM Histidine/Histidine chloride, 140 mM NaCl, pH 6.0, transferred into an optical 384-well plate by centrifugation through a 0.4 µm filter plate and covered with paraffine oil. The hydrodynamic radius is measured repeatedly by dynamic light scattering on a DynaPro Plate Reader (Wyatt) while the samples are heated with a rate of 0.05 °C/min from 25 °C to 80 °C. Alternatively, samples were transferred into a 10 µL 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 they were heated at a rate of 0.1 °C/min from 25°C to 90°C.

The aggregation onset temperature is defined as the temperature at which the hydrodynamic radius (DLS) or the scattered light intensity (Optim1000) starts to increase. The melting temperature is defined as the inflection point in a fluorescence intensity vs. wavelength graph.

5 Results are shown in **Tables 1 and 2**.

**Table 1:** VEGF and IL-1beta binding kinetics of 1HVL2.3 as assessed by SPR

| human IL-1beta |             |               |                        | human VEGF 121 |             |               |                        |
|----------------|-------------|---------------|------------------------|----------------|-------------|---------------|------------------------|
| ka<br>[1/Ms]   | kd<br>[1/s] | t1/2<br>[min] | K <sub>D</sub><br>[pM] | ka<br>[1/Ms]   | kd<br>[1/s] | t1/2<br>[min] | K <sub>D</sub><br>[pM] |
| 5.61E+06       | 2.56E-02    | 0.5           | 4565                   | 1.75E+06       | 1.45E-05    | 796           | 8                      |

**Table 2:** Thermal stability and hydrophobicity of 1HVL2.3

| Tagg (°C) | T <sub>m</sub> (°C) DLS | HIC (relative retention time) |
|-----------|-------------------------|-------------------------------|
| 75 (+/-1) | 87 (+/-1)               | 0.58                          |

#### 10 **Example 4:**

##### **Improvement of bispecific anti-VEGF/anti-IL-1beta Fab fragment 1HVL2.3**

As illustrated above, the bispecific anti-VEGF/anti-IL-1beta Fab fragment 1HVL2.3, while being highly stable, exhibits an affinity to IL-1beta in the nanomolar range as well as significant hydrophobicity. For treatment of ocular vascular diseases, which requires injection of the therapeutic into the eye, it is desirable to provide the therapeutic with a high affinity for the target antigen and in very high concentrations to increase durability of the therapeutic effect and to minimize inconveniences for the patient. For the intended purpose it is therefore desired to increase affinity and to reduce hydrophobicity of the antibody to assure solubility in isotonic buffer in high concentrations.

Consequently, for clinical application the antibody required further improvement, e.g. with respect to IL-1beta binding (particularly by improving the

off-rate) and reducing hydrophobicity. Several rounds of maturations were performed by introducing distinct amino acid substitutions in the VH and VL domain. During the maturations candidate antibodies derived from antibody 1HVL2.3 were screened and selected based on their desired properties with respect to yield, affinity, simultaneous antigen binding, hydrophilicity, stability, viscosity and other parameters.

Improved candidate antibodies 1HVL5.15, 1HVL12.85 and RO7200394 were selected from a plurality of tested candidate antibody molecules. Amino acid sequences of those improved bispecific anti-VEGF/anti-IL-1beta Fab fragments are identified in **Table 3**.

**Table 3:** Amino acid sequences of bispecific anti-VEGF/anti-IL-1beta Fab fragments 1HVL2.3, 1HVL5.15, 1HVL12.85 and RO7200394 (the numbers refer to the SEQ ID NOs as used herein)

|           | <b>VH</b> | <b>VL</b> | <b>heavy chain</b> | <b>light chain</b> |
|-----------|-----------|-----------|--------------------|--------------------|
| 1HVL2.3   | 1         | 2         | 9                  | 10                 |
| 1HVL5.15  | 21        | 22        | 24                 | 25                 |
| 1HVL12.85 | 11        | 12        | 18                 | 19                 |
| RO7200394 | 11        | 12        | 20                 | 19                 |

**Figures 2 and 3** illustrate an alignment of the variable heavy chain domains and the variable light chain domains of the generated bispecific anti-VEGF/anti-IL-1beta Fab fragments. Numbering of the amino acid positions within the VH and VL domains is according to the Kabat numbering system. For simplicity, the numbering is included in the Figure, further illustrating framework and CDR amino acid positions.

#### **Example 5:**

#### **Antigen binding kinetics of improved bispecific anti-VEGF/anti-IL-1beta Fab fragments**

Binding kinetics to VEGF and IL-1beta for the candidate antibodies were assessed as described in Example 3 using the indicated bispecific anti-VEGF/anti-IL-1beta Fab fragments (amino acid sequence as illustrated in **Table 3**). For

comparison, antigen binding kinetics of prior art anti-VEGF/anti-IL-1beta antibody 0032, a full length IgG antibody, as disclosed in WO2016/075034 are depicted.

Results of IL-1beta binding kinetics is shown in **Table 4** and **Table 5**.

- 5 **Table 4:** Human IL-1beta binding kinetics of bispecific anti-VEGF/anti-IL-1beta antibodies as assessed by SPR (data for prior art antibody 0032 from WO2016/075034)

|           |     | human IL-1beta |          |            |                     |
|-----------|-----|----------------|----------|------------|---------------------|
| antibody  |     | ka [1/Ms]      | kd [1/s] | t1/2 [min] | K <sub>D</sub> [pM] |
| 0032      | IgG | 2.49E+06       | 3.05E-04 | 38         | 120                 |
| 1HVL2.3   | Fab | 5.61E+06       | 2.56E-02 | 0.5        | 4565                |
| 1HVL5.15  | Fab | 1.66E+06       | 1.07E-04 | 109 ± 13*  | 59 ± 8*             |
| 1HVL12.85 | Fab | 4.76E+06       | 1.35E-04 | 86         | 28                  |
| RO7200394 | Fab | 4.73E+06       | 1.02E-04 | 114        | 21                  |

\*n=4

- 10 **Table 5:** Cynomolgus IL-1beta binding kinetics of bispecific anti-VEGF/anti-IL-1beta antibodies as assessed by SPR

|           |     | cynomolgus IL-1beta |          |            |                     |
|-----------|-----|---------------------|----------|------------|---------------------|
| antibody  |     | ka [1/Ms]           | kd [1/s] | t1/2 [min] | K <sub>D</sub> [pM] |
| 1HVL2.3   | Fab | 3.14E+06            | 1.82E-02 | 0.6        | 5794                |
| 1HVL5.15  | Fab | 2.60E+06            | 1.18E-04 | 98         | 45                  |
| 1HVL12.85 | Fab | 3.34E+06            | 6.54E-05 | 177        | 20                  |
| RO7200394 | Fab | 2.97E+06            | 7.94E-05 | 146        | 27                  |

Binding kinetics to IL-1beta of other species and related proteins was assessed for antibodies 1HVL5.15 and 1HVL12.85 by SPR using the same

experimental setup as described in Example 3. No binding was observed towards rat IL-1beta, pig IL-1beta, human IL-1alpha and human IL-1RA. Weak binding was observed towards murine and rabbit IL-1-beta.

Results of VEGF binding kinetics is shown in **Table 6**.

- 5 **Table 6:** Human VEGF121 binding kinetics of bispecific anti-VEGF/anti-IL-1beta antibodies as assessed by SPR (data for prior art antibody 0032 from WO2016/075034)

|           |     | human VEGF121 |          |            |                     |
|-----------|-----|---------------|----------|------------|---------------------|
| antibody  |     | ka [1/Ms]     | kd [1/s] | t1/2 [min] | K <sub>D</sub> [pM] |
| 0032      | IgG | 2.77E+04      | <1E-06   |            | <100                |
| 1HVL2.3   | Fab | 1.75E+06      | 1.45E-05 | 796        | 8                   |
| 1HVL5.15  | Fab | 1.53E+06      | 1.47E-05 | 789        | 10                  |
| 1HVL12.85 | Fab | 2.50E+06      | 1.51E-05 | 764        | 6                   |
| RO7200394 | Fab | 2.85E+06      | 1.63E-05 | 707        | 6                   |

Simultaneous antigen binding of the candidate antibodies to VEGF and IL-1beta was assessed as follows:

- 10 An anti-His capturing antibody (GE Healthcare 28995056) was immobilized to a Series S Sensor Chip C1 (GE Healthcare 29104990) using standard amine coupling chemistry resulting in a surface density of approximately 500 resonance units (RU). As running and dilution buffer, HBS-P+ (10 mM HEPES, 150 mM NaCl pH 7.4, 0.05% Surfactant P20) was used. Human VEGF121-His was captured to the
- 15 surface followed by consecutive injections of the candidate antibodies and IL-1beta. The surface was regenerated by injecting 10 mM Glycine pH 1.5 for 60s. Bulk refractive index differences were corrected by subtracting blank injections and by subtracting the response obtained from the control flow cell without captured human VEGF121.

- 20 Simultaneous binding of the candidate antibodies to VEGF and IL-1beta was confirmed for all improved bispecific anti-VEGF/anti-IL-1beta Fab fragments, i.e. 1HVL5.15, 1HVL12.85 and RO7200394. **Figure 4** illustrates simultaneous binding

of anti-VEGF/anti-IL-1beta 1HVL12.85. **Figure 5** illustrates simultaneous binding of anti-VEGF/anti-IL-1beta RO7200394.

Simultaneous antigen binding of full length IgG prior art antibody 0032 (WO2016/075034) was also assessed using the same experimental setup. The results are shown in **Figure 6**.

**Example 6:**

**Inhibition of binding of VEGF and IL-1beta to respective receptors**

***Receptor Inhibition Assay***

Inhibition of binding of VEGF and IL-1beta to their respective receptors, hVEGFR2 and IL-1betaR1, in presence of candidate antibody RO7200394 (Fab fragment) was assessed as described below. For comparison, kinetics of prior art anti-VEGF/anti-IL-1beta antibody 0032 (full length IgG), as disclosed in WO2016/075034, were assessed as well.

hVEGFR2 (R&D Systems 357-KD) and IL-1bR1 (Sino Biological Inc. 10126-H02H) were immobilized on different flow cells to a Series S Sensor Chip CM5 (GE Healthcare 29104988) using standard amine coupling chemistry resulting in surface densities of approximately 8000 and 20000 resonance units (RU), respectively. As running and dilution buffer, HBS-P+ (10 mM HEPES, 150 mM NaCl pH 7.4, 0.05% Surfactant P20) was used.

For assessing VEGF-receptor binding inhibition, a final concentration of 200 nM of RO7200394 or 400 nM antibody 0032 was preincubated with 50 nM VEGF121. For assessing IL-1beta-receptor binding inhibition, a final concentration of 200 nM of RO7200394 or 200 nM antibody 0032 was with preincubated with 50 nM IL-1beta. Samples were diluted (1:2) in the corresponding 50 nM VEGF121 or IL-1beta solution.

The antibody/ligand mixtures were injected onto the VEGFR2 or IL-1R1 surface for 60s at a flow rate of 5 µl/min. After a dissociation phase for 60s, the VEGFR2 surface was regenerated by injecting 5 mM NaOH for 60s, whereas the IL-1R1 surface was regenerated by injecting 10 mM Glycine pH 3.0 followed by 5 mM NaOH for 60s each. Bulk refractive index differences were corrected by subtracting blank injections and by subtracting the response obtained from the blank control flow cell. For evaluation, the binding response 5 seconds after inject end was taken. The derived response in RU was transformed to a binding response relative to the initial

signal corresponding to the ligand(s) without antibody. IC50 values were calculated using a 4 parameter logistic model (XLfit, ID Business Solutions Ltd.)

Results are shown in **Table 7** and **Table 8**; and **Figure 7A** (VEGFR2 inhibition in presence of antibody RO7200394), **Figure 7B** (VEGFR2 inhibition in presence of antibody 0032), **Figure 8A** (IL-1R1 inhibition in presence of antibody RO7200394) and **Figure 8B** (IL-1R1 inhibition in presence of antibody 0032).

**Table 7:** VEGFR2 binding inhibition of bispecific anti-VEGF/anti-IL-1beta antibodies without (- IL-1beta) or in presence of IL-1beta (+ IL-1beta)

| antibody         |     | IC50<br>(-IL-1beta) | IC50<br>(+IL-1beta) |
|------------------|-----|---------------------|---------------------|
| <b>0032</b>      | IgG | 67 nM               | 62 nM               |
| <b>RO7200394</b> | Fab | 44 nM               | 39 nM               |

**Table 8:** IL-1betaR1 binding inhibition of bispecific anti-VEGF/anti-IL-1beta antibodies without (- VEGF) or in presence of VEGF (+ VEGF)

| antibody         |     | IC50<br>(-VEGF) | IC50<br>(+VEGF) |
|------------------|-----|-----------------|-----------------|
| <b>0032</b>      | IgG | 34 nM           | 34 nM           |
| <b>RO7200394</b> | Fab | 22 nM           | 24 nM           |

It is demonstrated that binding of IL-1beta to the bispecific anti-VEGF/anti-IL-1beta Fab fragment of the invention does not interfere with inhibition of the VEGF/VEGFR2 interaction. Also, binding of VEGF to the bispecific anti-VEGF/anti-IL-1beta Fab fragment of the invention does not interfere with inhibition of the IL-1beta/IL-1betaR1 interaction.

#### ***VEGF competition ELISA***

The following buffers were used: PBS (1x PBS pH7.4); PBST (1x PBS supplemented with 0.1% v/v Tween-20); PBST 1% BSA (PBST supplemented with 1% BSA (Bovine Serum Albumin solution 30% from Sigma-Aldrich, A0336)); NaHCO<sub>3</sub> (NaHCO<sub>3</sub> solution, pH 9.4, made from BupH Buffer Packs

(ThermoScientific, 28382)); 2% MPBST (PBST supplemented with 2% (w/v) skimmed milk powder (Carl Roth, T145.3)).

96-well-Plates (Maxisorp Nunc-Immoplates) were coated with 50µL/well rhVEGFR-1-Fc (R&D #321-FL-050) at a final concentration of 1 µg/ml in  
5 200 mM NaHCO<sub>3</sub>, pH 9.4 for 1 hour at room temperature.

Meanwhile, antibody Fab fragments of different concentrations were preincubated with VEGF for form an antibody Fab-VEGF premix as follows: A dilution series of antibody Fab fragment was prepared by adding 280 µl of a solution of antibody Fab fragments (409.6 nM antibody in PBST-1% BSA) into the wells of the first column  
10 of a round bottom 96-well PS plate. The individual wells of columns 2-12 of the same plate were filled with 140 µl PBST-1% BSA. Subsequently, 1:2 dilutions were performed by transferring 140 µl of the antibody solution into the next column, thorough mixing and proceed with transferring 140 µl of this diluted antibody solution to the next colum. This is repeated until column 11. Excess of 140µl were  
15 discarded so that all wells comprise 140 µl. Column 12 served as the control (blank). For preincubation with VEGF, round bottom 96-well PS plates were prefilled with 50 µl/well of a 2 nM VEGF121 (Humanzyme, HZ-1206, Lot 0614-01) or a 2 nM VEGF165 (Humanzyme, HZ-1153, Lot 0716-01) solution in PBST-1%BSA. 50µl of the dilution series of the respective antibody Fab fragment were added to the  
20 VEGF121, or VEGF165, respectively; mixed thoroughly and incubated for 1.5 hours at room temperature.

The rhVEGFR-1-Fc coated plates were washed two times with PBST and blocked for 45 min with 200µl of 2% MPBST. After washing off the MPBST solution twice with PBST, 50µl of the antibody Fab-VEGF premix were added to the plate and  
25 incubated for 1.5 hours at room temperature. Subsequently, the plates were washed twice with PBST. Then, 50µl of a solution comprising biotinylated anti-VEGF antibody (R&D, BAF293; 1:2000 dilution in PBST) and SA-HRP (KPL, 14-30-00; 1:2000 dilution in PBST) was added and incubated for 30 min at room temperature. After 6x washing with PBST, 50µl of TMB substrate solution (two-component HRP  
30 substrate (KPL, 34021); used at room temperature) was added and incubated for 30 min at room temperature. 50 µl of 1N H<sub>2</sub>SO<sub>4</sub> was added and absorbance was read at 450nm.

Results are shown in **Figure 9** and **Figure 10** and illustrate improvement of VEGF-R1 binding inhibition of 1HVL12.85 and RO7200394 over 1HVL2.3.

**Example 7:****Biophysical properties of improved bispecific anti-VEGF/anti-IL-1beta Fab fragments (stability and hydrophobicity)**

Indicated biophysical properties of the candidate antibodies were assessed as described in Example 3 using the indicated bispecific anti-VEGF/anti-IL-1beta Fab fragments (amino acid sequence as illustrated in **Table 3**).

**Table 9** illustrates the thermal stability and hydrophobicity of the analysed antibodies. For comparison, the thermal stability of prior art anti-VEGF/anti-IL-1beta antibody 0032, a full length IgG antibody, as disclosed in WO2016/075034 is included. Chromatograms from HIC are shown in **Figure 11** for the bispecific anti-VEGF/anti-IL-1beta Fab fragments and in **Figure 12** for prior art anti-VEGF/anti-IL-1beta IgG antibody 0032.

**Table 9:** Thermal stability and hydrophobicity of bispecific anti-VEGF/anti-IL-1beta antibodies (data for prior art antibody 0032 from WO2016/075034)

| antibody  |     | Tagg (°C) | Tm (°C)<br>DLS | HIC<br>(relative<br>retention<br>time) |
|-----------|-----|-----------|----------------|----------------------------------------|
| 0032      | IgG | 55        | 62.5           | 0.81                                   |
| 1HVL2.3   | Fab | 75 (+/-1) | 87 (+/-1)      | 0.58                                   |
| 1HVL12.85 | Fab | 73 (+/-1) | 85 (+/-1)      | 0.03                                   |
| RO7200394 | Fab | 73 (+/-1) | 85 (+/-1)      | 0.04                                   |

**Example 8:****Biophysical properties of improved bispecific anti-VEGF/anti-IL-1beta Fab fragments (dynamic viscosity)**

The viscosity of the candidate antibodies was assessed as follows using the indicated bispecific anti-VEGF/anti-IL-1beta Fab fragments (amino acid sequence as illustrated in **Table 3**):

Viscosity was measured with the latex-bead DLS method as described before (He F et al.; Anal Biochem. 2010 Apr 1;399(1):141-3). In brief, samples were concentrated to >200 mg/mL (based on material availability) with centrifugal concentration devices, e.g. Amicon Ultra – 0.5 mL Centrifugal Filters, Ultracel – 10K, Cat.-No. UFC501096.

A dilution series from approximately 10 mg/mL to the maximal feasible concentration was prepared and Polysorbate 20 and beads (Nanosphere Size Standards, Nom Diam: 300nm, 1% solids, ThermoFisher Cat.-No.3300A) were added to a final concentration of 0.02% (PS20) and 0.03% (w/w, beads), respectively.

A small aliquot of these samples was centrifuged for 1 minute at maximum speed before the protein concentration was determined by UV280 absorption.

The remaining sample was transferred to an optical 384-well plate, covered with a layer of paraffin oil to prevent evaporation, and DLS data were recorded at the indicated temperature. From the DLS data for apparent hydrodynamic radius of the latex beads, the viscosity of the solution was calculated as described in He F et al.; *supra*. The viscosity at the highest concentration measured is reported in **Table 10**. Results are also shown in **Figure 13** (1HVL12.85) and **Figure 14** (RO7200394).

**Table 10:** Viscosity of bispecific anti-VEGF/anti-IL-1beta antibodies at 15°C

| antibody         |     | Viscosity           |
|------------------|-----|---------------------|
| <b>1HVL12.85</b> | Fab | 18.4 cP @ 195 mg/ml |
| <b>RO7200394</b> | Fab | 14.6 cP @ 210 mg/ml |

Results indicate that antibodies of the invention may be formulated in high concentrations comprising a viscosity below the acceptable viscosity limit for syringeability, which is up to 30 cP. While both tested antibodies are shown to be highly concentratable, the effect is more prominent in the RO7200394 antibody.

In consequence, the antibodies of the invention are highly suitable for ocular application as they allow for provision of a high molar dose in a limited injection

volume, which when combined with high potency results in a high durability and consequently, a reduced dosing frequency, which is desirable to reduce difficulties on the patient's side.

**Example 9:**

5     **Chemical stability of improved bispecific anti-VEGF/anti-IL-1beta Fab fragment**

**Chemical degradation test:**

Antibody samples were formulated in 20 mM His/HisCl, 140 mM NaCl, pH 6.0, and were split into three aliquots: one aliquot was re-buffered into PBS, respectively, and two aliquots were kept in the original formulation. The PBS aliquot and one His/HisCl aliquot were incubated for 2 weeks (2w) at 40°C (His/NaCl) or 37°C (PBS) in 1 mg/ml, the PBS sample was incubated further for total 4 weeks (4w). The third control aliquot sample was stored at -80°C. After incubation ended, samples were analyzed for relative active concentration (Biacore; active concentration of both stressed aliquots of each binder is normalized to unstressed 4°C aliquot), aggregation (SEC) and fragmentation (capillary electrophoresis or SDS-PAGE) and compared with the untreated control.

**Binding activity after stress was assessed as follows:**

Anti-Fab capturing antibody (GE Healthcare 28958325) was immobilized on a Series S Sensor Chip CM5 (GE Healthcare 29104988) using standard amine coupling chemistry resulting in a surface density of 4000 – 6000 resonance units (RU). As running and dilution buffer, HBS-P+ (10 mM HEPES, 150 mM NaCl pH 7.4, 0.05% Surfactant P20) was used. Antibody anti-VEGF/anti-IL-1beta antibody 1HVL12.85 having a concentration of 2 µg/ml was injected for 60s at a flow rate of 5 µl/min. HuVEGF121 (in house preparation) or huIL-1 beta (Peprotech 200-01B) at a concentration of 2 µg/ml each was injected for 60s, dissociation was monitored for 60s at a flow rate of 5 µl/min. The surface was regenerated by two consecutive injections of 10 mM Glycine pH 2.1 for 60s each. Bulk refractive index differences were corrected by subtracting blank injections and by subtracting the response obtained from the blank control flow cell. For evaluation, the binding response 5 seconds after inject end was taken. To normalize the binding signal, the VEGF or IL-1 beta binding response was divided by the anti-Fab response. The relative active concentration was calculated by referencing each temperature stressed sample to the corresponding, non-stressed sample.

Results are shown in Tables 11 and 12.

**Table 11:** Binding activity after stress for anti-VEGF/anti-IL-1beta antibody 1HVL12.85 (amino acid sequence see **Table 3**)

| stress conditions | Binding activity after stress IL-1beta [%binding] | Binding activity after stress VEGF [%binding] |
|-------------------|---------------------------------------------------|-----------------------------------------------|
| 2w/ 40°C / pH 6.0 | 98                                                | 98                                            |
| 2w/ 37°C / pH 7.4 | 98                                                | 96                                            |
| 4w/ 40°C / pH 6.0 | 97                                                | 95                                            |
| 4w/ 37°C / pH 7.4 | 97                                                | 94                                            |

**Table 12:** Molecular integrity after stress for anti-VEGF/anti-IL-1beta antibody 1HVL12.85 (amino acid sequence see **Table 3**)

| stress conditions | Aggregation [%aggregates SEC] | Fragmentation [%fragments CE-SDS] |
|-------------------|-------------------------------|-----------------------------------|
| 2w/ 40°C / pH 6.0 | 0.98                          | 1.68                              |
| 2w/ 37°C / pH 7.4 | 1.94                          | 3.39                              |

#### **Example 10:**

#### **Structural analysis of improved bispecific anti-VEGF/anti-IL-1beta Fab fragment 1HVL5.15**

Structural analysis of anti-VEGF/anti-IL-1beta Fab fragment 1HVL5.15 was performed by x-ray crystallography as follows:

#### ***Complex formation and crystallization of the ternary complex IL1 $\beta$ -VEGF121 - Fab 1HVL5.15***

For complex formation, antibody 1HVL5.15 Fab fragment and human IL1 $\beta$  (Peprotech) were mixed in a 1:1.1 molar ratio. After incubation for 16 hours overnight at 4°C, human VEGF121 (in house preparation) was added to obtain a ternary complex which was concentrated to 10mg/ml. Initial crystallization trials were performed in sitting drop vapor diffusion setups at 21°C. First micro-crystals appeared within 4 days out of 1.4M sodium malonate. Subsequent seeding experiments yielded crystals out of 0.1 M sodium cacodylate pH 5.5, 0.1 M calcium acetate, 12% PEG8000. The crystals were directly harvested from the screening plate without any further optimization steps.

***Data collection and structure determination***

For data collection crystals were flash cooled at 100K in precipitant solution with addition of 15% ethylene glycol as cryoprotectant. Diffraction data were collected at a wavelength of 1.0000 Å using a PILATUS 6M detector at the beamline X10SA of the Swiss Light Source (Villigen, Switzerland). Data have been processed with XDS (Kabsch, W. *Acta Cryst.* **D66**, 133-144 (2010)) and scaled with SADABS (BRUKER). The crystals belong to the space group C222<sub>1</sub> with cell axes of a= 177.97 Å, b= 286.70 Å, c= 105.39 Å,  $\alpha=\beta=\gamma=90^\circ$  and diffract to a resolution of 2.97Å. The structure was determined by molecular replacement with PHASER (McCoy, A.J. et al. *J. Appl. Cryst.* **40**, 658-674 (2007)) using the coordinates of a related in house structures of a Fab fragment, IL1 $\beta$  and pdb entry 1MKK for VEGF as search models. Programs from the CCP4 suite (Collaborative Computational Project, Number 4 *Acta Cryst.* **D50**, 760-763 (1994)) and Buster (Bricogne, G., et al. (2011). Buster version 2.9.5 Cambridge, United Kingdom : Global Phasing Ltd) have been used for refinement of the structure. Manual rebuilding of protein using difference electron density was done with COOT (Emsley, P., et al. *Acta Cryst* **D66**, 486-501 (2010)). Data collection and refinement statistics are summarized in **Table 13**. All graphical presentations were prepared with PYMOL (DeLano Scientific, Palo Alto, CA, 2002). The structure was analyzed with the program CONTACT from the CCP4 suite (Collaborative Computational Project, Number 4 *Acta Cryst.* **D50**, 760-763 (1994)) to identify paratope and epitope residues using a contact distance maximum of 4 Å.

**Table 13:** Data collection and structure refinement statistics (x-ray crystallography)

| <b>Data Collection</b>      |                              |
|-----------------------------|------------------------------|
| Wavelength (Å)              | 1.0                          |
| Resolution <sup>1</sup> (Å) | 49.76 - 2.97 (3.07 - 2.97)   |
| Space group                 | C222 <sub>1</sub>            |
| Unit cell (Å, °)            | 177.97, 286.70, 105.39 , 90° |
| Unique reflections          | 55924 (5206)                 |
| Multiplicity                | 7.60 (7.76)                  |
| Completeness (%)            | 99.9 (99.9)                  |
| Mean I/ $\sigma$ (I)        | 6.53 (0.84)                  |
| R-meas                      | 0.25 (0.93)                  |

|                                    |                            |
|------------------------------------|----------------------------|
| <b>Data Collection</b>             |                            |
| CC1/2                              | 0.999 (0.297)              |
| <b>Refinement</b>                  |                            |
| Resolution <sup>1</sup> (Å)        | 49.76 - 2.97 (3.02 - 2.97) |
| Reflections used in refinement     | 55851 (2753)               |
| Reflections used for R-free        | 2595 (109)                 |
| R-work <sup>3</sup>                | 0.204 (0.262)              |
| R-free <sup>4</sup>                | 0.252 (0.287)              |
| Number of atoms                    | 10540                      |
| Protein residues                   | 577                        |
| RMS bonds (Å)                      | 0.010                      |
| RMS angles (°)                     | 1.40                       |
| Ramachandran favored (%)           | 95.32                      |
| Ramachandran outliers (%)          | 0.15                       |
| Rotamer outliers (%)               | 0.84                       |
| Clashscore                         | 9.03                       |
| Average B-factor (Å <sup>2</sup> ) | 72.98                      |
| protein                            | 72.98                      |

<sup>1</sup> Values in parentheses refer to the highest resolution bins.

<sup>2</sup>  $R_{\text{merge}} = \sum |I - \langle I \rangle| / \sum I$  where I is intensity.

<sup>3</sup>  $R_{\text{work}} = \sum |F_o - \langle F_c \rangle| / \sum F_o$  where  $F_o$  is the observed and  $F_c$  is the calculated structure factor amplitude.

<sup>4</sup>  $R_{\text{free}}$  was calculated based on 5% of the total data omitted during refinement.

Amino acid residues in contact with the respective antigens, VEGF and IL-1beta, were identified from the crystal structure of the bispecific anti-VEGF/anti-IL-1beta Fab fragment 1HVL5.15 in complex. An illustration of the position of paratope amino acid residues within the VH and VL domains is depicted in **Figure 2 and Figure 3**.

Amino acids from light chain CDR1 and CDR3 as well as heavy chain CDR2 contribute to the VEGF paratope. The VEGF paratope does not comprise amino acids from light chain CDR2, heavy chain CDR1 and heavy chain CDR3. The IL-1beta paratope does not comprise amino acids from light chain CDR2.

- 5 The amino acid residues identified to contribute to antigen binding are identified in **Table 14** (for the variable heavy chain domain amino acid residues) and **Table 15** (for the variable light chain domain amino acid residues). Amino acid positions are numbered according to the Kabat numbering system (the same numbering is used in **Figures 2 and 3**). Amino acids positions involved in antigen binding are identified by their Kabat position in the VH or VL domain (see also the numbering in **Figures 2 and 3**).

**Table 14:** Variable heavy chain amino acid residues involved in antigen binding as identified by crystal structure analysis of bispecific anti-VEGF/anti-IL-1beta antibody 1HVL5.15

| VH     | VEGF                       | IL-1beta        |
|--------|----------------------------|-----------------|
| FR1    | -                          | 2, 26, 28, 30   |
| H-CDR1 | -                          | 31, 35b, 35c    |
| FR2    | -                          | -               |
| H-CDR2 | 55, 56, 58, 61, 62, 63, 64 | 52a             |
| FR3    | 66, 83                     | 94              |
| H-CDR3 | -                          | 95, 96, 98, 101 |
| FR4    | -                          | -               |

- 15 **Table 15:** Variable light chain amino acid residues involved in antigen binding as identified by crystal structure analysis of bispecific anti-VEGF/anti-IL-1beta antibody 1HVL5.15

| VL     | VEGF              | IL-1beta       |
|--------|-------------------|----------------|
| FR1    | 2                 | -              |
| L-CDR1 | 27, 27a, 27c, 27d | 32             |
| FR2    | -                 | 49             |
| L-CDR2 | -                 | 50, 53, 54, 56 |
| FR3    | 67, 68, 69        | 57             |
| L-CDR3 | 92, 93, 94, 96    | 91             |
| FR4    | -                 | -              |

**PATENT CLAIMS**

1. An antibody that binds to human VEGF and to human IL-1beta, comprising a  
5 VEGF paratope and an IL-1beta paratope within one cognate pair of a variable  
light chain domain (VL domain) and a variable heavy chain domain (VH  
domain), wherein the VEGF paratope comprises amino acid residues from CDR-  
H2, CDR-L1 and CDR-L3 of the antibody, wherein the IL-1beta paratope  
10 comprises amino acid residues from the CDR-H1, CDR-H3 and CDR-L2 of the  
antibody.
2. The antibody of one of one of the preceding claims, wherein the antibody  
comprises a VH domain comprising (a) CDR-H1 comprising the amino acid  
15 sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence  
of SEQ ID NO:14, and (c) CDR-H3 comprising the amino acid sequence of SEQ  
ID NO:15, and a VL domain comprising (d) CDR-L1 comprising the amino acid  
sequence of SEQ ID NO:16, (e) CDR-L2 comprising the amino acid sequence of  
SEQ ID NO:17, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID  
20 NO:8.
3. The antibody of one of one of the preceding claims, wherein the antibody  
comprises a VH domain comprising (a) CDR-H1 comprising the amino acid  
sequence of SEQ ID NO:13, (b) CDR-H2 comprising the amino acid sequence  
of SEQ ID NO:14, (c) CDR-H3 comprising the amino acid sequence of SEQ ID  
25 NO:15, (d) a human heavy chain framework with (i) FR1 comprising amino acid  
residues E2, G26, V28, and K30, (ii) FR3 comprising amino acid residues R66,  
R83, and K94; and a VL domain comprising (e) CDR-L1 comprising the amino  
acid sequence of SEQ ID NO:16, (f) CDR-L2 comprising the amino acid  
sequence of SEQ ID NO:17, (g) CDR-L3 comprising the amino acid sequence  
30 of SEQ ID NO:8, and (h) a human light chain framework with (i) FR1 comprising  
amino acid residue I2, (ii) FR2 comprising amino acid residue Y49, (iii) FR3  
comprising amino acid residues G57, E67, D68, and Q69, wherein the numbering  
of the VH and VL domains is according to the Kabat numbering system.
4. The antibody of one of the preceding claims, comprising a VH domain  
35 comprising amino acid residues E2, G26, V28, K30, W31, N35b, D35c, K52a,  
D55, H56, Y58, T61, K62, F63, I64, R66, R83, K94, D95, V96, F98, and D101,  
and a VL domain comprising amino acid residues I2, Y27, W27a, S27c, S27d,

L32, Y49, D50, Y53, K54, L56, G57, E67, D68, Q69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the VH and VL domains is according to the Kabat numbering system.

- 5        5. The antibody of claim 4, comprising
- a VEGF paratope comprising the following amino acid residues in the VH domain D55, H56, Y58, T61, K62, F63, I64, R66, and R83, and the following amino acid residues in the VL domain I2, Y27, W27a, S27c, S27d, E67, D68, 10        Q69, R92, Y93, H94, and Y96; and
  - an IL-1beta paratope comprising the following amino acid residues in the VH domain E2, G26, V28, K30, W31, N35b, D35c, K52a, K94, D95, V96, F98, and D101, and the following amino acid residues in the VL domain L32, Y49, D50, Y53, K54, L56, G57, Y91.
- 15
6. The antibody of any one of the preceding claims, comprising (a) a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11; and (b) a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid 20        sequence of SEQ ID NO:12.
7. The antibody of any one of the preceding claims, comprising (a) a VH domain comprising an amino acid sequence of SEQ ID NO:11 with up to 15 amino acid substitutions; and (b) a variable light chain domain comprising an amino acid 25        sequence of SEQ ID NO:12 with up to 15 amino acid substitutions.
8. The antibody of any one of the preceding claims, comprising a VH sequence of SEQ ID NO:11 and a VL sequence of SEQ ID NO:12.
- 30        9. An antibody that specifically binds to human VEGF and to human IL-1beta, comprising a VH sequence of SEQ ID NO:11 and a VL sequence of SEQ ID NO:12.
- 35        10. The antibody of any one of the preceding claims, wherein an antibody Fab fragment of the antibody binds (i) to human VEGF121 with a  $K_D$  of less than 10 pM as measured by surface plasmon resonance, and (ii) to human IL-1beta with a  $K_D$  of less than 30 pM as measured by surface plasmon resonance.

11. The antibody of any one of the preceding claims, wherein an antibody Fab fragment of the antibody exhibits an aggregation onset temperature of more than 70 °C.
- 5 12. The antibody of any one of the preceding claims, wherein an antibody Fab fragment of the antibody exhibits a melting temperature of more than 80 °C as measured by dynamic light scattering.
- 10 13. The antibody of any one of the preceding claims, wherein the antibody is a bispecific antibody fragment.
14. An isolated nucleic acid encoding the antibody of any of claims 1 to 13.
- 15 15. A host cell comprising the nucleic acid of claim 14.
16. An expression vector comprising the nucleic acid of claim 15.
- 20 17. A method of producing an antibody that binds to human VEGF and to human IL-1beta comprising culturing the host cell of claim 15 so that the antibody is produced.