

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

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

10 In one aspect the disclosure 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 example the disclosure 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 20 simultaneously binds to human VEGF and human IL-1beta.

In one example the disclosure 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- 25 1beta paratope.

In one example the disclosure 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 30 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.

In one example the disclosure 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 example the disclosure 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.

In one example the disclosure 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.

In one example the disclosure 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.

In one example the disclosure 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.

In one example the disclosure 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.

5 In one example the disclosure 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,
10 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 example 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,
15 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 one example the disclosure provides an antibody that binds to human
20 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, S67, H68, E69, Y91, R92, Y93, H94, and Y96, wherein the numbering of the
25 VH and VL domains is according to the Kabat numbering system. In one example 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 example the disclosure provides an antibody that binds to human
35 VEGF and to human IL-1beta, 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.

5 In one example the disclosure 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
10 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 example the disclosure 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
20 (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
25 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 example the disclosure 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
35 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.

In one example the disclosure 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 example 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 example the disclosure 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 example the disclosure 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.

The 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 disclosure provides 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.

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

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

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

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

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

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

The disclosure provides the antibody produced by the method of the disclosure.

15 The disclosure provides a pharmaceutical formulation comprising the antibody of the disclosure and a pharmaceutically acceptable carrier.

The disclosure provides the antibody of the disclosure for use as a medicament, in one example for use in the treatment of a vascular disease.

20 The disclosure provides the use of the antibody of the disclosure or the pharmaceutical composition of the disclosure in the manufacture of a medicament, in one example a medicament for the treatment of a vascular disease.

The disclosure provides a method of treating an individual having a vascular disease comprising administering to the individual an effective amount of the antibody of the disclosure or the pharmaceutical composition of the disclosure.

25 The disclosure provides a method of inhibiting angiogenesis in an individual comprising administering to the individual an effective amount of the antibody of the disclosure or the pharmaceutical composition of the disclosure to inhibit angiogenesis

According to the disclosure 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 disclosure provides several valuable properties that allow its therapeutic application, like high affinity, hydrophilicity, and high stability. The antibody of the disclosure can be provided in high concentrations liquid formulations with a viscosity suitable for ocular application. The antibody of the disclosure 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.