IL-6 ANTIBODIES AND FUSION CONSTRUCTS AND CONJUGATES THEREOF

INCORPORATION BY REFERENCE TO ANY PRIORITY APPLICATIONS

[0001] Any and all applications for which a foreign or domestic priority claim is identified in the Application Data Sheet as filed with the present application are hereby incorporated by reference under 37 CFR §1.57.

SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled KDIAK044.txt, created March 1, 2019 which is 395,430 bytes in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

BIOLOGICAL SAMPLE DEPOSIT STATEMENT

[0003] In some embodiments, anti-IL-6 antibodies, fusions, and conjugates thereof are provided, which deposited in the American Type Culture Collection (ATCC), in accordance with the Budapest Treaty, under the numbers ____, on _____.

[0004] These deposits are made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of the deposit for 30 years from date of deposit. The deposit will be made available by the ATCC under the terms of the Budapest Treaty, and subject to an agreement between Applicant and the ATCC, which assures permanent and unrestricted availability of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the deposit to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. § 122 and the Commissioner's Rules pursuant thereto (including 37 C.F.R. § 1.14). Availability of the deposited

biological material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any Government in accordance with its Patent Laws.

FIELD

[0005] The present invention relates generally to fusion constructs that bind to IL-6 and/or VEGF.

BACKGROUND

[0006] Vascular endothelial growth factor A (VEGF-A) is a signal protein that mediates pro-angiogenic functions such as endothelial cell survival, proliferation, migration, and cell-cell permeability. Its activity has been shown to promote progression of retinal diseases such as choroidal neovascularization (CNV) in Age Related Macular Degeneration (AMD) and Diabetic Macular Edema (DME). Inhibition of VEGF-A signaling has been proven to be an effective means to stop progression of neovascular retinal diseases (Ferrara et al, Retina, 2006). Various therapeutic molecules have been developed to inhibit VEGF function. Among these, anti-VEGF monoclonal antibodies such as Ranibizumab and Bevacizumab have been shown to be safe and effective treatments against pathological angiogenesis. More recently, recombinantly made VEGFR fusion proteins such as Aflibercept (Eylea) and Conbercept (China), which act as VEGF "traps," are proving to be more effective and longer lasting than their antibody competitors.

[0007] Inflammation has been implicated in the pathogenesis of retinal diseases, and anti-inflammatory therapies such as steroids have been effective in treating uveitis and diabetic macular edema (DME). Detailed studies looking at inflammation and infection in the eye have shown that the pro-inflammatory cytokine, interleukin-6 (IL-6) is significantly elevated in the ocular fluids of refractory/chronic uveitis patients, and inhibition of IL-6 in animal models inhibits the onset of uveitis. High ocular fluid levels of IL-6 are also found in patients with DME and retinal vein occlusion. Additionally, chronic inflammatory cells have been seen on the surface of the Bruch's membrane in eyes with neovascular AMD, and patients with AMD have been reported to have increased serum levels of IL-6. Interestingly, IL-6 has also been observed to stimulate defective angiogenesis. In addition to autoimmune disorders such as rheumatoid arthritis, anti-IL-6 treatment has been shown to effectively treat uveitis and uveitic macular edema.

SUMMARY

[0008] In some ~~aspects~~embodiments, an isolated antagonist antibody that specifically binds to IL-6 that is conjugated to a polymer is provided.

[0009] In some ~~aspects~~embodiments, an isolated antagonistic antibody is provided. The antibody comprises a heavy chain amino acid variable region that comprises a heavy chain in Table 1A; and a light chain amino acid variable region that comprises the light chain in Table 1B.

[0010] In some ~~aspects~~embodiments, an isolated antagonist antibody is provided. The antibody comprises: a heavy chain variable region (VH) comprising a VH complementarity determining region one (CDR1), VH CDR2, and VH CDR3 of the VH having an amino acid sequence selected from the group consisting of the CDRs in Table 1A; and a light chain variable region (VL) comprising a VL CDR1, VL CDR2, and VL CDR3 of the VL having an amino acid sequence selected from the group consisting of the CDRs in Table 1B.

[0011] In some ~~aspects~~embodiments, an isolated antagonist antibody that binds to IL-6 is provided. The antibody comprises at least one of the following mutations based on EU numbering: L234A, L235A, and G237A.

[0012] In some ~~aspects~~embodiments, an isolated antagonistic antibody that binds to IL-6 is provided. The antibody comprises a CDR_H1 that is the CDR_H1 in Table 1A; a CDR_H2 that is the CDR_H2 in Table 1A; a CDR_H3 that is the CDR_H3 in Table 1A: a CDR_L1 that is the CDR_L1 in Table 1B; a CDR_L2 that is the CDR_L2 in Table 1B; a CDR_L3 that is the CDR_L3 in Table 1B; at least one of the following mutations (EU numbering): L234A, L235A, and G237A; and at least one of the following mutations (EU numbering): Q347C or L443C.

[0013] In some ~~aspects~~embodiments, a fusion protein is provided that comprises the isolated antagonist antibody; and a fragment of a VEGF binding protein.

[0013a] Disclosed herein is a fusion protein comprising an isolated antagonist antibody that specifically binds to IL-6; and a VEGF Trap, wherein the VEGF Trap is positioned either at an N-terminal end of a heavy chain comprising IL-6 VH; or between a hinge region and a CH1 domain of the heavy chain comprising IL-6 VH.

[0014] In some ~~aspects~~embodiments, a conjugate comprising any of the isolated antagonistic antibodies herein and a polymer is provided. The polymer is covalently attached to the antibody.

[0015] In some ~~aspects~~embodiments, a conjugate is provided. The conjugate comprises the fusion protein as provided herein and a polymer, wherein the polymer is covalently attached to the fusion protein.

[0015a] Disclosed herein is a conjugate comprising the fusion protein as defined herein; and a polymer, wherein the polymer is covalently attached to a heavy chain of the isolated antagonist antibody.

[0016] In some ~~aspects~~embodiments, an isolated cell line that produces the isolated antagonistic antibody or the fusion protein is provided.

[0016a] Disclosed herein is an isolated cell line that produces the fusion protein as defined herein.

[0017] In some ~~aspects~~embodiments, an isolated nucleic acid encoding an isolated antagonistic antibody or the fusion protein is provided.

[0017a] Disclosed herein is an isolated nucleic acid encoding the fusion protein as defined herein.

[0018] In some ~~aspects~~embodiments, a recombinant expression vector is provided. The vector comprises a nucleic acid encoding any of these constructs provided herein.

[0018a] Disclosed herein is a recombinant expression vector comprising the nucleic acid as defined herein.

[0019] ~~In some aspects,~~ Disclosed herein is a host cell comprising an expression vector as provided herein ~~is provided~~.

[0020] In some ~~aspects~~embodiments, a method of producing an IL-6 antagonist antibody or fusion protein thereof is provided. The method comprises: culturing a cell line that recombinantly produces an isolated antagonistic antibody or the fusion protein under conditions wherein the antibody is produced; and recovering the antibody.

[0020a] Disclosed herein is a method of producing a fusion protein, the method comprising: culturing a cell line that recombinantly produces the fusion protein as defined herein under conditions wherein the antibody is produced; and recovering the antibody.

[0021] In some ~~aspects~~embodiments, a pharmaceutical composition is provided. It comprises an isolated antagonistic antibody or the fusion protein and/or a conjugate as provided herein, and a pharmaceutically acceptable carrier.

[0021a] Disclosed herein is a pharmaceutical composition comprising the fusion protein as defined herein and/or the conjugate as defined herein, and a pharmaceutically acceptable carrier.

[0022] In some ~~aspects~~embodiments, a method for the treatment or prophylaxis of a disease in a patient in need thereof is provided. The method comprises administering to the patient an isolated antagonist antibody or the fusion protein and/or a conjugate as provided herein.

[0023] In some ~~aspects~~embodiments, a method for the treatment or prophylaxis of a disease in a patient in need thereof is provided. The method comprises identifying a patient having hyperactive IL-6 and/or VEGF activity; and administering to the patient an isolated antagonist antibody or the fusion protein and/or a conjugate as provided herein.

[0023a] Disclosed herein is the use of the fusion protein and/or the conjugate as defined herein in the manufacture of a medicament for the treatment or prophylaxis of a neovascular retinal disease in a patient in need thereof.

[0023b] Disclosed herein is the use of the fusion protein and/or the conjugate as defined herein in the manufacture of a medicament for the treatment or prophylaxis of an ocular disorder in a patient in need thereof.

[0024] In some ~~aspects~~embodiments, a fusion protein is provided that comprises an IL-6 VH, an IL-6 VL, an IL-6 Fc, and a VEGF Trap, wherein the VEGF Trap is fused to IL-6 in one of the following manners: 1) to an N-terminal end of a heavy chain comprising IL-6 VH; or 2) between a hinge region and after a CH1 domain of a heavy chain comprising IL-6 VH.

[0025] In some ~~aspects~~embodiments, an isolated antagonistic IL-6 antibody is provided that comprises a heavy chain amino acid variable region that comprises a heavy chain that has a sequence of at least one of SEQ ID NOs: 7-13,19-27, 89, 90, 256-262; and a light chain amino acid variable region that comprises the light chain that has a sequences of at least one of SEQ ID NOs: 91-93, 28-30.

[0026] In some ~~aspects~~embodiments, an isolated antagonist IL-6 antibody is provided that comprises: a heavy chain variable region (VH) comprising 3 complementarity determining regions: VH (CDR1), VH CDR2, and VH CDR3 having an amino acid sequence from the CDRs listed in SEQ ID NO: 256; and a light chain variable region (VL) comprising a VL CDR1, VL

CDR2, and VL CDR3 having an amino acid sequence selected from the group of CDRs listed in SEQ ID NO: 91-93.

[0027] In some ~~aspects~~embodiments, an isolated antagonist antibody that binds to IL-6 is provided. The antibody comprises at least one of the following mutations based on EU numbering: L234A, L235A, and G237A.

[0028] In some embodiments, an isolated antagonistic antibody that binds to IL-6 is provided and comprises: a CDR_H1 that is a CDR_H1 in SEQ ID NO: 172; a CDR_H2 that is a CDR_H2 in SEQ ID NO: 173; a CDR_H3 that is a CDR_H3 in SEQ ID NO: 174: a CDR_L1 that is a CDR_L1 in SEQ ID NO: 199; a CDR_L2 that is a CDR_L2 in SEQ ID NO: 200; a CDR_L3 that is a CDR_L3 in SEQ ID NO: 201; at least one of the following mutations (EU numbering): L234A, L235A, and G237A; and at least one of the following mutations (EU numbering): Q347C or L443C.

[0029] In some ~~aspects~~embodiments, a fusion protein comprising: the isolated antagonist antibody of any one provided herein (including fragments thereof)) and VEGF Trap is provided.

[0030] In some ~~aspects~~embodiments, a conjugate comprising: any of the isolated antagonistic antibodies (including fragments) provided herein; and a polymer is provided, wherein the polymer is covalently attached to the antibody.

[0031] In some ~~aspects~~embodiments, a conjugate comprising the fusion protein as provided herein; and a polymer, wherein the polymer is covalently attached to the fusion protein is provided.

[0032] In some ~~aspects~~embodiments, a VEGFR-Anti-IL-6 dual inhibitor is provided. The VEGFR-Anti-IL-6 dual inhibitor comprises a trap antibody fusion of an anti-IL 6 antibody and an anti-VEGF trap (VEGFR1/2), wherein the dual inhibitor includes at least one point mutation within a VEGFR sequence to reduce cleavage of the VEGFR protein.

[0033] In some ~~aspects~~embodiments, a protein construct comprising: at least 3 heavy chain CDRs; at least 3 light chain CDRs; a VEGF trap sequence; and a linker sequence, wherein each of the sequences is selected from a corresponding sequence within FIGs. 18-25 or from corresponding sequences from the tables herein (including, for example, 0.1A, 0.1B, 1A, 1B, 0.2A, and 0.2B).

[0034] In some ~~aspects~~embodiments, a fusion protein comprising an IL-6 VH; an IL-6 VL; an IL-6 Fc; and a VEGF Trap is provided, wherein the fusion protein alters HUVEC

proliferation. In some embodiments, altering HUVEC proliferation is inhibiting VEGF/IL6 mediated proliferation.

[0035] In some ~~aspects~~embodiments, a fusion protein comprising a sequence that is at least 80% identical to SEQ ID NO: 263 and at least 80% identical to SEQ ID NO: 117 is provided, wherein the fusion protein is further conjugated to a polymer.

[0036] In some ~~aspects~~embodiments, an isolated antagonist antibody that binds to IL-6 is provided. The antibody comprises at least one of the following mutations based on EU numbering: L234A, L235A, and G237A.

[0037] In some ~~aspects~~embodiments, an isolated antagonistic antibody that binds to IL-6, the antibody comprising: a CDR_H1 that is a CDR_H1 in SEQ ID NO: 172; a CDR_H2 that is a CDR_H2 in SEQ ID NO: 173; a CDR_H3 that is a CDR_H3 in SEQ ID NO: 174: a CDR_L1 that is a CDR_L1 in SEQ ID NO: 199;a CDR_L2 that is a CDR_L2 in SEQ ID NO: 200; a CDR_L3 that is a CDR_L3 in SEQ ID NO: 201;at least one of the following mutations (EU numbering): L234A, L235A, and G237A; and at least one of the following mutations (EU numbering): Q347C or L443C is provided.

[0038] In some ~~aspects~~embodiments, the VEGFR-Anti-IL-6 dual inhibitor comprises an anti-IL-6 heavy chain variable region sequences selected from SEQ ID NO: 7-13, 89, 90, and/or 256-262.

[0039] In some ~~aspects~~embodiments, the VEGFR-Anti-IL-6 dual inhibitor has a VEGF trap sequences selected from at least one of SEQ ID Nos: 145, 15, 16, or 17.

[0040] In some ~~aspects~~embodiments, the linker sequence is SEQ ID NO: 18.

[0041] In some ~~aspects~~embodiments, a heavy chain sequence for the Anti-IL-6 molecule is provided that is selected from at least one of SEQ ID NOs 19-27 or includes at least the sequence in one of SEQ ID NOs: 89, 90, 256-262.

[0042] In some ~~aspects~~embodiments, a light chain sequence for the anti-IL-6 molecule is provided that comprises at least 1, 2, or 3 light chain CDRs from at least one of SEQ ID NOs 76-84.

[0043] In some ~~aspects~~embodiments, a heavy chain sequence for the Anti-IL-6 molecule is provided that comprises at least 1, 2, or 3 heavy chain CDRs from at least one of SEQ ID NOs 49-75.

[0044] In some ~~aspects~~embodiments, a VEGFR-Anti-IL-6 dual inhibitor is provided that comprises a VEGFR-Fc sequence from at least one of SEQ ID NOs 85-88.

[0045] In some ~~aspects~~embodiments, a VEGFR-Anti-IL-6 dual inhibitor is provided that comprises one or more of the sequences in any one or more of SEQ ID Nos 7-13, 145, 15-17, 18-84.

[0046] In some ~~aspects~~embodiments ~~embodiments~~, a VEGFR-Anti-IL-6 dual inhibitor is provided that comprises an IL-6 VH; an IL-6 VL; an IL-6 Fc; a VEGF Trap; and a linker. In some embodiments, the IL-6 VH comprises a sequence from an IL6 VH sequence in any one of SEQ ID NOs 19-27, 31-39, 89, 90, or 256-262. In some embodiments, the IL-6 VL comprises a sequence from an IL6 VL sequence in any one of SEQ ID Nos 28-30 or 91-93. In some embodiments, the Fc comprises a sequence from a Fc sequence in any one of SEQ ID NOs 40-48. In some embodiments, the VEGF Trap comprises a sequence from a VEGF trap sequence in any one of SEQ ID NOs: 145, 15, 16, or 17.

[0047] In some ~~aspects~~embodiments, a fusion protein is provided that comprises an IL-6 VH; an IL-6 VL; an IL-6 Fc; and a VEGF Trap, wherein the fusion protein alters HUVEC proliferation. In some embodiments, altering HUVEC proliferation is inhibiting VEGF/IL6 mediated proliferation.

[0048] In some ~~aspects~~embodiments, a fusion protein is provided that comprises a sequence that is at least 80% identical to SEQ ID NO: 263 and at least 80% identical to SEQ ID NO: 117 is provided. The fusion protein is further conjugated to a polymer. In some embodiments, the fusion protein is at least 95% identical to SEQ ID NO: 263 and at least 95% identical to SEQ ID NO: 117. In some embodiments, the protein comprises at least a) SEQ ID NO: 172, 173, 174, 199, 200, and 201, or b) a substitutions of 1, 2, or 3 amino acids within SEQ ID NO: 172, 173, 174, 199, 200, and/or 201, wherein the substitution is a conservative substitution.

[0049] In some ~~aspects~~embodiments, an antibody of any one of provided herein comprises a) an amino acid sequence of SEQ ID NO: 169 and 170 or b) an amino acid sequence that is at least 80% identical to SEQ ID NO: 169 and at least 80% identical to SEQ ID NO: 170.

[0050] In some ~~aspects~~embodiments, a fusion protein of any one provided herein comprises a) an amino acid sequence of SEQ ID NO: 169 and 170 or b) an amino acid sequence that is at least 80% identical to SEQ ID NO: 169 and at least 80% identical to SEQ ID NO: 170.

[0051] In some ~~aspects~~embodiments, a conjugate of any one provided herein comprises a) an amino acid sequence of SEQ ID NO: 169 and 170 or b) an amino acid sequence that is at least 80% identical to SEQ ID NO: 169 and at least 80% identical to SEQ ID NO: 170.

[0052] In some ~~aspects~~embodiments, a VEGFR-Anti-IL-6 dual inhibitor of any one provided herein comprises a) an amino acid sequence of SEQ ID NO: 169 and 170 or b) an amino acid sequence that is at least 80% identical to SEQ ID NO: 169 and at least 80% identical to SEQ ID NO: 170.

[0053] In some ~~aspects~~embodiments, a protein of any one provided herein comprises a) an amino acid sequence of SEQ ID NO: 169 and 170 or b) an amino acid sequence that is at least 80% identical to SEQ ID NO: 169 and at least 80% identical to SEQ ID NO: 170.

BRIEF DESCRIPTION OF THE DRAWINGS

[0054] FIG. 1 depicts a sequence of IL-6.

[0055] FIG. 2A shows Compound L.

[0056] FIG. 2B shows Compound K.

[0057] FIG. 2C shows the synthesis of OG1802 from R3707.

[0058] FIG. 2D shows OG1786.

[0059] FIG. 2E shows the synthesis of OG1546 from OG1550.

[0060] FIG. 2F shows the synthesis of OG1784 from OG1546 and OG1563.

[0061] FIG. 2G shows the synthesis of OG1405 from OG1784.

[0062] FIG. 2H shows the synthesis of OG 1785 from OG1405.

[0063] FIG. 2I shows the synthesis of OG1786 from OG1785.

[0064] FIG. 2J shows OG1801.

[0065] FIG. 2K shows OG1802.

[0066] FIG. 2L shows Compound E.

[0067] FIG. 3 depicts a flow chart for antibody selection and optimization.

[0068] FIG. 4 depicts ELISA data showing that anti-IL-6 mAb binds to IL-6, but not an IL-6/IL-6R complex, and that IL-6/IL-6R complex formation is inhibited by anti-IL-6 mAb.

[0069] FIG. 5 depicts some embodiments of the heavy and light chain variable regions of an IL-6-Ab. Embodiments of CDRs are shown in boxed regions. These sequences can also be employed in a IL-6 Ab-VEGF Trap fusion construct.

[0070] FIG. 6 depicts some embodiments of an IL-6-VEGF Trap fusion protein. The VEGF Trap domains are positioned at either at the N-terminus immediately preceding the variable domain (left) or positioned between the Fab region and the hinge region of the antibody (right).

[0071] FIG. 7 depicts sensorgrams and table that demonstrate dual inhibitor molecules VEGFR-AntiIL-6 and AntiIL-6-VEGFR bind with similar affinity to VEGF-A as the anti-VEGF antibody OG1950 and Eylea. Thus, the position of the VEGF trap does not alter its affinity for its target.

[0072] FIG. 8 depicts sensorgrams of independent or combined VEGF-A and IL-6 binding to dual inhibitors. IL-6Sensorgrams of mixed targets were compared to a theoretical curve (sum of individual IL-6 and VEGF-A sensorgrams). Results show theoretical and experimental curves superpose, which qualitatively indicates that both targets can bind to the dual inhibitor molecule without influencing each other's binding.

[0073] FIG. 9 depicts OD450nm the results from various ELISA IL-6 assays. In the bridging ELISA (top), both dual inhibitors bridged btVEGF to IL-6, indicating both configurations can bind to both targets. EC50 VEGF Trap-antiIL-6 = 0.079 nM and antiIL-6-VEGF Trap = 0.026 nM. Eylea and anti-IL-6 served as a negative controls. FIG. 9 also shows the results of an IL-6/IL-6R complex ELISA (middle). Dual inhibitors and antiIL-6 inhibited IL-6/IL-6R complex formation to the same degree. IC50 values: anti-IL-6 = 0.36 nM, VEGF Trap-antiIL-6 = 0.47 nM, and antiIL-6-VEGF Trap = 0.32 nM. Eylea served as a negative control. FIG. 9 also shows the results of a VEGF/VEGFR competitive ELISA (bottom). Dual inhibitors, Eylea, and OG1950 inhibited VEGF binding to VEGFR to varying degrees. Eylea and the antiIL-6-VEGF Trap construct are comparable (4.24 nM vs. 4.53 nM), while the VEGF Trap-antiIL-6 construct was ~2 fold better (1.74 nM), and OG1950 showed superior maximal inhibition to other inhibitors (1.55 nM).

[0074] FIG. 10 depicts some embodiments of a method for preparing an antibody conjugate (which can also be applied for an Ab-Trap or Trap-Ab conjugate as well). While depicted as an antibody, one of skill in the art will appreciate, in the present context, that the Ab depiction within FIG. 10 can be swaped with a trap fusion (as shown in FIG. 6). For the sake of simplicity, the generic antibody depicted herein represents both options as an antibody and options as a fusion arrangement in the fusion trap context (unless, of course, it is already depicted as a fusion).

[0075] FIGs. 11A-11B depict SDS-PAGE bands of SeeBlue®Plus2 standard, Anti-IL-6, Anti-IL-6-VEGFR, and VEGFR-Anti-IL-6. FIG. 11C depicts the conjugate construct of VEGFR-Anti-IL6, which is a fusion of Anti-VEGF (VEGFR1/2) and Anti-IL-6 conjugated with a phosphorylcholine-based polymer.

[0076] FIG. 12 depicts results of transfer to a PVDF membrane, N-terminal Edman Sequencing, which show that the cleaved products share the same N-Terminal sequence (LTHRQT), which shows that the cleavage site is located at the VEGF trap region.

[0077] FIGs. 13A-B depict SDS-PAGE bands of SeeBlue®Plus2 standard and 19 VEGF trap constructs.

[0078] FIGs. 13C-13E depict the sequence listings of VEGF_trap_variant_1, VEGF_trap_variant_2, and VEGF_trap_variant_3.

[0079] FIGs. 13F-13G depict SDS-PAGE bands of SeeBlue®Plus2 standard and 4 VEGF trap variants, including VEGFR_variant_3, which has double point mutations T94I and H95I.

[0080] FIGs. 14A-14F depict Biacore assay results and measurements of affinity to VEGF-A.

[0081] FIG. 15 depicts a cell-based VEGF stimulated VEGFR reporter assay.

[0082] FIG. 16A depicts an assay of inhibition of VEGF/IL6 mediated human umbilical vein endothelial cells ("HUVEC") tubule formation.

[0083] FIGs. 16B-16C depict statistics of the tubule formation assays with different parameters.

[0084] FIG. 17 depicts the results of an HUVEC proliferation assay.

[0085] FIG. 18 illustrates embodiments of Anti-IL-6 heavy chain variable region sequences. CDRs are underlined.

[0086] FIG. 19 illustrates various embodiments of VEGF trap sequences. Section that varies between the sequences are in bold and underlined.

[0087] FIG. 20 illustrates some embodiments of linker (GS) sequence embodiments. It can be present as a double repeat Gly-Gly-Gly-Gly-Ser linker (GS).

[0088] FIG. 21 illustrates some embodiments of heavy chain sequence for Anti-IL-6 molecules. CDRs are underlined. FIG. 21 includes both FIG. 21A and FIG. 21B, which provide various embodiments of CDRs within the noted sequences.

[0089] FIG. 22 illustrates some embodiments of light chain sequences for Anti-IL-6 molecules. CDRs are underlined. FIG. 22 includes both FIG. 22A and FIG. 22B, which provide various embodiments of CDRs within the noted sequences.

[0090] FIG. 23 illustrates some embodiments of heavy chain sequences for Anti-IL-6 molecules. CDRs are underlined. FIG. 23 includes both FIG. 23A and FIG. 23B, which provide various embodiments of CDRs within the noted sequences.

[0091] FIGs. 24A-24B illustrate some embodiments of combinations of CDRs of FIGs. 21-23.

[0092] FIG. 25 illustrates some embodiments of VEGFR-Fc sequence variants. Section that varies between the sequences are in bold and underlined.

[0093] FIGs. 26A-26C illustrate affinity binding data.

[0094] FIG. 27 depicts the sequences of some embodiments of the VEGFR-AntiIL6-sequences. The CDRs (as defined by Kabat) are underlined. The greyed sections indicate the VEGFR constructs. The bolded text indicates the linker section. Mutations L234A, L235A, G237A and L443C (EU numbering) are double underlined. Each of these sections can be exchanged for other corresponding sections provided herein (e.g., alternative linkers or CDRs, etc.)

[0095] FIG. 28 depicts a SDS-PAGE of VEGFR-AntiIL6 reduction (Cys – decapping) reaction products. The lanes are as follows: 1. VEGFR-AntiIL6; 2. VEGFR-AntiIL6 – fully reduced (TCEP); 3. Novex sharp pre-stained protein standard; 4. VEGFR-AntiIL6 + 30x TCEP, initial point; 5. VEGFR-AntiIL6 + 30x TCEP, after 30 min; 6. VEGFR-AntiIL6 + 30x TCEP, after 60 min; 7. VEGFR-AntiIL6 TCEP treated, buffer exchanged; 8. VEGFR-AntiIL6 + 15x dHAA, initial point; 9. VEGFR-AntiIL6 + 15x dHAA, after 30 min; 10. VEGFR-AntiIL6 + 15x dHAA, after 60 min; 11.VEGFR-AntiIL6 decapped; 12. VEGFR-AntiIL6 decapped, fully reduced (TCEP); Gel: NuPAGE Bis-Tris 4-12% Protein amount: 4 µg / lane; 30x TCEP = 30 times molar excess TCEP 15x dHAA = 15 times molar excess dHAA

[0096] FIG. 29 depicts a SDS-PAGE of VEGFR-AntiIL6-OG1802 conjugate CEX chromatography. The Non-reducing gel: NuPAGE Bis-Tris 4-12%. Buffer A: 20 mM Sodium Acetate pH 5.5. Buffer B: 20 mM Sodium Acetate pH 5.5, 500 mM NaCl. The lanes are as follows: 1. VEGFR-AntiIL6; 2. VEGFR-AntiIL6-OG1802 (load); 3. Novex sharp pre-stained protein standard; 4. Flow-through; 5. Chase; 6. 30% buffer B – aliquot 1; 7. 30% buffer B

– aliquot 2; 8. 30% buffer B – aliquot 3; 9. 30% buffer B – aliquot ; 10. 40% buffer B – aliquot 1; 11. 40% buffer B – aliquot 2; 12. 40% buffer B – aliquot 3; 13. 40% buffer B – aliquot 4; 14. 60% buffer B – aliquot 1; 15. 60% buffer B – aliquot 2; 16. 60% buffer B – aliquot 3; 17. 100% buffer B – strip. Lanes 6-13 show protein conjugated material, conjugate cannot penetrate gel due to large size. Lanes 14-17 show protein mixture possibly containing aggregated, conjugated and non-conjugated protein material

[0097] FIG. 30 depicts a SDS-PAGE of a protein-polymer conjugate vs non-conjugate protein material. Gel analysis demonstrates 57% heavy chain and 96% light chain band intensity ratios when bioconjugate (lane 3) is compared to VEGFR-AntiIL6 reference standard (lane 2), which indicates the presence of one OG1802 polymer per VEGFR-AntiIL6 molecule Reducing gel is : NuPAGE Bis-Tris 4-12% Reducing gel is : NuPAGE Bis-Tris 4-12%.

[0098] FIG. 31 depicts a SEC-MALS of VEGFR-AntiIL6-OG1802. The molecular weight of VEGFR-AntiIL6 conjugate was determined with integrated size exclusion chromatography (Shodex-SB806M-HQ) and light scattering (MALS). Top panel. Chromatogram shows the presence of a single eluting peak. Absence of additional peaks and shoulder suggest no aggregates and degraded material were present after conjugation and subsequent CEX separation steps. Bottom panel. Protein conjugate analysis of selected peak showed an experimentally measured average molecular weight (Mw) of 983 kDa for the VEGFR-AntiIL6-OG1802 bioconjugate. This value results from the conjugation of one VEGFR-AntiIL6 molecule (Mw ~ 189 kDa) and one OG1802 polymer (Mw ~ 794 kDa).

[0099] FIG. 32 depicts the results of a VEGFR-AntiIL6 CEX chromatography. Gel: is Novex 8-16% Tris-Glycine (reducing conditions), M = SeeBlue®Plus2 standard Lanes D12-F11 correspond to samples aliquoted at different buffer B concentrations as indicated on chromatogram Intact (I) and cleaved (C) heavy chains are indicated. In Column Porus XS Buffer A is 20 mM Sodium Phosphate pH 6 and buffer B is 20 mM Sodium Phosphate pH 6, 1 M NaCl.

[0100] FIG. 33 depicts the results of a VEGFR-AntiIL6 HIC chromatography. Gel: is Novex 8-16% Tris-Glycine / reducing conditions, M = SeeBlue®Plus2 standard. L = VEGFR-AntiIL6 intact and cleaved mixture (load). Remaining lanes correspond to samples aliquoted at different buffer B concentrations as indicated on chromatogram. Intact (I) and cleaved (C) heavy chains are indicated . In Column Hi Trap Butly HP Buffer A is : 20 mM Sodium Phosphate pH 6, 1 M Ammonium sulfate. Buffer B is: 20 mM Sodium Phosphate pH 6.

[0101] FIG. 34 depicts the results of a VEGF/VEGFR competitive ELISA.

[0102] FIG. 35 depicts the results of a IL6/IL6R complex ELISA.

[0103] FIG. 36 depicts the results of a cell based VEGF stimulated VEGFR reporter assay.

[0104] FIG. 37 depicts the lipopolysaccharide stimulated tubule formation in HUVECs.

[0105] FIG. 38 depicts the lipopolysaccharide stimulated tubule formation in HUVECs

[0106] FIG. 39 depicts lipopolysaccharide stimulated tubule formation in HUVECs

[0107] FIG. 40A depicts the inhibition of VEGF/IL6 mediated proliferation of HUVECs.

[0108] FIG. 40B depicts the inhibition of HUVEC proliferation with increasing number of cells per well.

DETAILED DESCRIPTION

[0109] To directly reduce the concurrent inflammation and defective angiogenesis that drive pathogenesis of neovascular retinal pathologies, presented herein are designed molecules that simultaneously block the functions of the pro-inflammatory cytokine IL-6 and the pro-angiogenic signal protein VEGF. These molecules are comprised of (1) an anti-IL-6 monoclonal antibody fused to (2) two VEGF binding domains of VEGF receptors (VEGFRs). The anti-IL-6 moiety specifically binds IL-6 and inhibits its interaction with the IL-6 receptor (IL-6R). The VEGF Trap moiety includes of a fusion of two VEGF binding domains (VEGFR1 domain 2, VEGFR2 domain 3) that work as a VEGF trap, preventing VEGF from binding to VEGF receptors. Additionally, in some embodiments, each of these dual inhibitor molecules is equipped with an unpaired cysteine at its C-terminus which can be conjugated with a half-life extending phosphorylcholine based biopolymer.

[0110] Various embodiments provided herein will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second

edition (Sambrook et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-1998) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J.D. Capra, eds., Harwood Academic Publishers, 1995).

[0111] The following terms, unless otherwise indicated, shall be understood to have the following meanings: the term "isolated molecule" as referring to a molecule (where the molecule is, for example, a polypeptide, a polynucleotide, or an antibody) that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other molecules from the same source, e.g., species, cell from which it is expressed, library, etc., (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or expressed in a cellular system different from the system from which it naturally originates, will be "isolated" from its naturally associated components. A molecule also may be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

[0112] As used herein, unless designated otherwise, the term "IL-6" or "IL6" refers to human IL-6. In some embodiments, other forms of IL-6 are contemplated, and will be designated by specific reference to the other organisms, e.g., canine, feline, equine, and bovine. One exemplary human IL-6 is found as UniProt Accession NumberP05231.

[0113] Anti-IL-6 antibodies or other biologics described herein are typically provided in isolated form. This means that an antibody is typically at least 50% w/w pure of interfering proteins and other contaminants arising from its production or purification but does not exclude the possibility that the antibody is combined with a pharmaceutically acceptable excipient intended to facilitate its use. Sometimes antibodies are at least 60, 70, 80, 90, 95 or 99% w/w pure of interfering proteins and contaminants from production or purification. Often an antibody (or antibody conjugate) is the predominant macromolecular species remaining after its purification.

[0114] An "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also, unless otherwise specified, any antigen binding portion thereof that competes with the intact antibody for specific binding, fusion proteins comprising an antigen binding portion, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site. Antigen binding portions include, for example, Fab, Fab', $F(ab')_2$, Fd, Fv, domain antibodies (dAbs, e.g., shark and camelid antibodies), fragments including complementarity determining regions (CDRs), single chain variable fragment antibodies (scFv), maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0115] A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chains each consist of four framework regions (FRs) connected by three complementarity determining regions (CDRs) also known as hypervariable regions, and contribute to the formation of the antigen binding site of antibodies. If variants of a subject variable region are desired, particularly with substitution in amino acid residues outside of a CDR region (i.e., in the framework region), appropriate amino acid substitution, preferably, conservative amino acid substitution, can be identified by comparing the subject variable region to the variable regions of other antibodies which contain CDR1 and CDR2 sequences in the same canonincal class as the subject variable region (Chothia and Lesk, J Mol Biol 196(4): 901-917, 1987).

[0116] In certain embodiments, definitive delineation of a CDR and identification of residues comprising the binding site of an antibody is accomplished by solving the structure of the antibody and/or solving the structure of the antibody-ligand complex. In certain embodiments, that can be accomplished by any of a variety of techniques known to those skilled in the art, such as X-ray crystallography. In certain embodiments, various methods of analysis can be employed to identify or approximate the CDR regions. In certain embodiments, various methods of analysis can be employed to identify or approximate the CDR regions. Examples of such methods include, but are not limited to, the Kabat definition, the Chothia definition, the IMGT approach (Lefranc et al., 2003) Dev Comp Immunol. 27:55-77), computational programs such as Paratome (Kunik et al., 2012, Nucl Acids Res. W521-4), the AbM definition, and the conformational definition.

[0117] The Kabat definition is a standard for numbering the residues in an antibody and is typically used to identify CDR regions. See, e.g., Johnson & Wu, 2000, Nucleic Acids Res., 28: 214-8. The Chothia definition is similar to the Kabat definition, but the Chothia definition takes into account positions of certain structural loop regions. See, e.g., Chothia et al., 1986, J. Mol. Biol., 196: 901-17; Chothia et al., 1989, Nature, 342: 877-83. The AbM definition uses an integrated suite of computer programs produced by Oxford Molecular Group that model antibody structure. See, e.g., Martin et al., 1989, Proc Natl Acad Sci (USA), 86:9268-9272; "AbM™, A Computer Program for Modeling Variable Regions of Antibodies," Oxford, UK; Oxford Molecular, Ltd. The AbM definition models the tertiary structure of an antibody from primary sequence using a combination of knowledge databases and ab initio methods, such as those

described by Samudrala et al., 1999, “Ab Initio Protein Structure Prediction Using a Combined Hierarchical Approach,” in PROTEINS, Structure, Function and Genetics Suppl., 3:194-198. The contact definition is based on an analysis of the available complex crystal structures. See, e.g., MacCallum et al., 1996, J. Mol. Biol., 5:732-45. In another approach, referred to herein as the “conformational definition” of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, e.g., Makabe et al., 2008, Journal of Biological Chemistry, 283:1156-1166. Still other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs may be defined in accordance with any of Kabat, Chothia, extended, IMGT, Paratome, AbM, and/or conformational definitions, or a combination of any of the foregoing.

[0118] As known in the art, a “constant region” of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

[0119] As used herein, "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, 1975, Nature 256:495, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated

using the techniques described in McCafferty et al., 1990, Nature 348:552-554, for example. As used herein, "humanized" antibody refers to forms of non-human (e.g. murine) antibodies that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Preferably, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. The humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance.

[0120] A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen binding residues.

[0121] The term “chimeric antibody” is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody. The term "epitope" refers to that portion of a molecule capable of being recognized by and bound by an antibody at one or more of the antibody's antigen-binding regions. Epitopes often consist of a surface grouping of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. In some embodiments, the epitope can be a protein epitope. Protein epitopes can be linear or conformational. In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. A "nonlinear epitope" or "conformational epitope" comprises noncontiguous polypeptides (or amino acids) within the antigenic protein to which an antibody specific to the epitope binds. The term "antigenic epitope" as used herein, is defined as a portion of an antigen to which an antibody can specifically bind as determined by any method well known in the art, for example, by conventional immunoassays. Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, e.g., using the techniques described in the present

specification. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct competition and cross-competition studies to find antibodies that compete or cross-compete with one another for binding to IL-6, e.g., the antibodies compete for binding to the antigen.

[0122] The term "compete," as used herein with regard to an antibody, means that a first antibody, or an antigen-binding portion thereof, binds to an epitope in a manner sufficiently similar to the binding of a second antibody, or an antigen-binding portion thereof, such that the result of binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to "cross-compete" with each other for binding of their respective epitope(s). Both competing and cross-competing antibodies are encompassed by the present invention. Regardless of the mechanism by which such competition or cross-competition occurs (e.g., steric hindrance, conformational change, or binding to a common epitope, or portion thereof), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

[0123] As used herein, an antibody "interacts with" IL-6 when the equilibrium dissociation constant is equal to or less than 20 nM, preferably less than about 6 nM, more preferably less than about 1 nM, most preferably less than about 0.75 nM. In some embodiments, the affinity of the antibody is between 400 and 800 pM, e.g., 450-700, or 500-600 pM.

[0124] An IL-6 antagonist antibody encompasses antibodies that block, antagonize, suppress or reduce (to any degree including significantly) a IL-6 biological activity such as binding to IL-6R, IL-6/IL-6R complex binding to gp130, phosphorylation and activation of Stat3, cell proliferation, and stimulation of IL-6 mediated inflammatory or pro-angiogenic pathways. For

purpose of the present disclosure, it will be explicitly understood that the term “IL-6 antagonist antibody” encompasses all the previously identified terms, titles, and functional states and characteristics whereby the IL-6 itself, an IL-6 biological activity, or the consequences of the biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree. In some embodiments, an IL-6 antagonist antibody binds IL-6. Examples of IL-6 antagonist antibodies are provided herein.

[0125] An antibody that “preferentially binds” or “specifically binds” (used interchangeably herein) to an epitope is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit “specific binding” or “preferential binding” if it reacts or associates more frequently, and/or more rapidly, and/or with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody “specifically binds” or “preferentially binds” to a target if it binds with greater affinity, and/or avidity, and/or more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to an IL-6 epitope is an antibody that binds this epitope with greater affinity, and/or avidity, and/or more readily, and/or with greater duration than it binds to other IL-6 epitopes or non-IL-6 epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

[0126] As used herein, “substantially pure” refers to material which is at least 50% pure (i.e., free from contaminants), more preferably, at least 90% pure, more preferably, at least 95% pure, yet more preferably, at least 98% pure, and most preferably, at least 99% pure.

[0127] A “host cell” includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

[0128] As known in the art, the term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a

variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3. As is known in the art, an Fc region can be present in dimer or monomeric form.

[0129] As used in the art, "Fc receptor" and “FcR” describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. FcRs are reviewed in Ravetch and Kinet, 1991, Ann. Rev. Immunol., 9:457-92; Capel et al., 1994, Immunomethods, 4:25-34; and de Haas et al., 1995, J. Lab. Clin. Med., 126:330-41. “FcR” also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., 1976, J. Immunol., 117:587; and Kim et al., 1994, J. Immunol., 24:249).

[0130] A “functional Fc region” possesses at least one effector function of a native sequence Fc region. Exemplary “effector functions” include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity; phagocytosis; down-regulation of cell surface receptors (e.g. B cell receptor), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g. an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

[0131] A “native sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, yet retains at least one effector function of the native sequence Fc region. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten

amino acid substitutions, and preferably, from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably, at least about 90% sequence identity therewith, more preferably, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity therewith.

[0132] As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results.

[0133] As used herein, "IL-6 and/or VEGF related disorders" include, for example, ocular disorders and systemic disorders. Ocular disorders include ocular disorders such as the ophthalmic inflammatory disease scleritis, non-proliferative diabetic retinopathy, proliferative diabetic retinopathy, diabetic macular edema, prevention of diabetic macular edema, prevention of proliferative diabetic retinopathy, wet age-related macular degeneration, prevention of wet age-related macular degeneration, dry age-related macular degeneration, venous, arterial or other blockage of the ocular and or retinal blood vessels with or without retinal edema, anterior and posterior uveitis, uveitic macular edema, and intraocular tumors. IL-6 related disorders also include disorders where there is an elevated level of IL-6 activity due to IL-6 interacting with IL-6R or soluble IL-6R (sIL-6R). In some embodiments, any one or more of the fusion proteins provided herein and/or any one or more of the conjugates provided herein can be used for treatment or prevention of any one or more of the IL-6 and/or VEGF related disorders. In some embodiments, the disorders include systemic diseases that affect the eye such as Grave's disease or neuromyelitis optica, or systemic diseases that do not affect the eye such as multiple sclerosis, rheumatoid arthritis. In some embodiments, the disorders include cytokine release syndrome following CAR-T or similar immune-oncology therapeutics.

[0134] Additionally, anti-IL6 molecules abrogate the induction of IL-6 expression observed following treatment with anti-PD-1/PD-L1 molecules (Tsukamoto et al, Cancer Res; 2018 78(17); 5011–22*)*. It has also been shown that VEGF signaling blockade can improve anti-PD-L1 treatment (Allen et al, Sci Transl Med 2017 April 12: 9(385)). Thus, these dual inhibitors can be used in combination with PD-1/PDL-1 modulators and/or other immune checkpoint inhibitors, to synergistically treat cancer. Other disorders can include cerebral edema in

glioblastoma where anti-IL6 therapy may show additional benefits to anti-VEGF treatments. Other disorders include those with solid tumors.

[0135] As used herein, "Ameliorating" means a lessening or improvement of one or more symptoms as compared to not administering an IL-6 antibody, IL-6 antibody-VEGF Trap fusion, conjugate of IL-6 antibody, and/or conjugate of IL-6 antibody-VEGF Trap fusion. "Ameliorating" also includes shortening or reduction in duration of a symptom.

[0136] As used herein, "VEGF Trap" or similar term denotes the VEGF binding domains (VEGFR1 domain 2, VEGFR2 domain 3). This fragment allows for the protein to work as a VEGF trap, preventing VEGF from binding to cellularly expressed VEGF receptors. An example of this sequence can be found in Table 1C. In some embodiments, the VEGF Trap only includes VEGFR1 domain 2, VEGFR2 domain 3. Various embodiments of Trap proteins are known in the art and can be found, for example in U.S. Pub. No. 20150376271, the entirety of which, with respect to various VEGF Trap embodiments (which are VEGFR proteins or fragments thereof) and fusions thereof, is incorporated herein by reference. In some embodiments, the term "VEGF Trap" or similar term refers to a full length extracellular region or any portion thereof, or combination of portions from different VEGF receptors that can antagonize signaling between at least one VEGF and VEGFR.

[0137] As used herein, "IL-6 antibody-VEGF Trap fusion", "IL-6 antibody-VEGF Trap", "Ab IL-6-VEGF Trap", "AntiIL-6-VEGF Trap", "VEGFR-AntiIL6", "VEGFR-AntiIL-6", "VEGF Trap-anti-IL6 Antibody Fusion (TAF)", "VEGF Trap-IL6", "VEGFR IL-6", "IL6-VEGFR" or similar term or inverse terms (e.g. "VEGF Trap-IL-6 Ab," "VEGF Trap-IL-6 antibody fusion," etc.) denote the fusion between the IL-6 antibody and the VEGF Trap. Embodiments are depicted in FIG. 6. When used generically, the order of the two terms can be swapped. When used specifically, the order of the two terms denotes the relative position of the components in the construct. the term "Ab-Trap", "IL-6 Ab-VEGF Trap" "Ab IL-6 VEGF Trap" or "Ab IL-6-Trap" or "antiIL-6 VEGF Trap", "Trap-Ab", AntiIL-6-VEGFR, AntiIL6-VEGFR or other similar term or inverse terms (e.g. "VEGF Trap-IL-6 Ab," "VEGF Trap-IL-6 antibody fusion," etc.) denotes the arrangement of the Ab fused to the relevant domains of a VEGF binding protein so as to provide a VEGF trap. As noted above, this section of the VEGF binding protein is one that prevents VEGF from binding to VEGF receptors. As described herein, the arrangement (ordering) of the Trap and antibody sections can be varied. Thus, unless denoted otherwise explicitly or by context, the

phrases used herein regarding Ab-Trap (or Il-6/VEGF Trap, etc.) fusions, denote all disclosed embodiments for the positioning of the antibody and the Trap. Thus, unless explained otherwise, the phrase Ab-Trap (or Il-6/VEGF Trap, etc.), denotes the left embodiment in FIG. 6, and the right embodiment in FIG. 6, and both embodiments in FIG. 6. Thus, the general language is denoted as disclosing all three options for convenience. If the orientation is specifically denoted, it can be denoted, for example, by stating that the "arrangement" can be one of: Trap-Ab, Trap IL-6 Ab, VEGF Trap Ab IL-6, VEGF Trap Ab IL6. Similarly, it will be appreciated that the context of some of the present Examples specific orientations or arrangements of the molecules, which are denoted by the context of the Example. Both arrangements (in the alternative and combined) are explicitly contemplated for all discussions of fusion proteins provided herein. In addition, due to the ordering, it is appreciated that the phrase IL-6 Ab, when used in the context of the fusion protein, includes both the option where the antibody is contiguous, FIG. 6, left-hand side, and where the TRAP is positioned "within" the Ab (FIG. 6 right-hand side). Again, the term "Ab" or "antibody", when used in the fusion protein context (or other similar term), encompasses all three options (left-hand side of FIG. 6, right-hand side of FIG. 6, and both options), unless otherwise noted. In some embodiments, the VEGF Trap is fused to IL-6 in one of the following manners: to an N-terminal end of a heavy chain comprising IL-6 VH; or between a hinge region and after a CH1 domain of a heavy chain comprising IL-6 VH. There is no difference between the designations of Ab, antibody, "anti'" or other similar term when used in a name to designate and antibody or fragment thereof. There is no difference between the designations of "Il-6" or "IL6" or "IL-6". As used herein, when referencing a fusion construct with IL-6, the terms "VEGF", "VEGFR", "VEGF Trap", "VEGFR Trap" are used interchangeably. The terms can have different meanings when used separately from the IL-6 fusion arrangement, which will depend upon the context of the term in question.

[0138] As used herein, the term "biopolymer" denotes that a polymer has been linked to the protein of interest. The term can also be described as the "conjugated" form of the protein. This can be done for all of the proteins described herein. Thus, IL-6 Ab biopolymers and IL-6 antibody-VEGF Trap biopolymers are contemplated for all such IL-6 Ab and IL-6 antibody-VEGF Trap provided herein. In addition, VEGF Trap biopolymers are also provided.

[0139] As used herein, "Antagonistic antibody" denotes an antibody that blocks one or more function or activity of the molecule that the antibody binds to.

[0140] As used herein, an "effective dosage" or "effective amount" of drug, compound, or pharmaceutical composition is an amount sufficient to effect any one or more beneficial or desired results. In more specific aspects, an effective amount prevents, alleviates or ameliorates symptoms of disease, and/or prolongs the survival of the subject being treated. For prophylactic use, beneficial or desired results include eliminating or reducing the risk, lessening the severity, or delaying the outset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as reducing one or more symptoms of a disease such as, for example, AMD including, for example without limitation, dry AMD and wet AMD, decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication, and/or delaying the progression of AMD in patients. An effective dosage can be administered in one or more administrations. For purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective dosage" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0141] Anti-IL-6 antibodies are administered in an effective regime meaning a dosage, route of administration and frequency of administration that delays the onset, reduces the severity, inhibits further deterioration, and/or ameliorates at least one sign or symptom of a disorder. If a patient is already suffering from a disorder, the regime can be referred to as a therapeutically effective regime. If the patient is at elevated risk of the disorder relative to the general population but is not yet experiencing symptoms, the regime can be referred to as a prophylactically effective regime. In some instances, therapeutic or prophylactic efficacy can be observed in an individual patient relative to historical controls or past experience in the same patient. In other instances, therapeutic or prophylactic efficacy can be demonstrated in a preclinical or clinical trial in a population of treated patients relative to a control population of untreated patients.

[0142] Anti-IL-6-VEGF Traps are administered in an effective regime meaning a dosage, route of administration and frequency of administration that delays the onset, reduces the severity, inhibits further deterioration, and/or ameliorates at least one sign or symptom of a disorder. If a patient is already suffering from a disorder, the regime can be referred to as a therapeutically effective regime. If the patient is at elevated risk of the disorder relative to the general population but is not yet experiencing symptoms, the regime can be referred to as a prophylactically effective regime. In some instances, therapeutic or prophylactic efficacy can be observed in an individual patient relative to historical controls or past experience in the same patient. In other instances, therapeutic or prophylactic efficacy can be demonstrated in a preclinical or clinical trial in a population of treated patients relative to a control population of untreated patients.

[0143] The “biological half-life” of a substance is a pharmacokinetic parameter which specifies the time required for one half of the substance to be removed from an organism following introduction of the substance into the organism.

[0144] The term “preventing” or “prevent” refers to (a) keeping a disorder from occurring (b) delaying the onset of a disorder or onset of symptoms of a disorder, or (c) slowing the progression of an existing condition. Unless denoted otherwise, “preventing” does not require the absolute prohibition of the event from occurring.

[0145] An “individual” or a "subject" is a mammal or bird, more preferably, a human. Mammals also include, but are not limited to, farm animals (e.g., cows, pigs, horses, chickens, etc.), sport animals, pets, primates, horses, dogs, cats, mice and rats.

[0146] As used herein, "vector" means a construct, which is capable of delivering, and, preferably, expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

[0147] As used herein, "expression control sequence" means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

[0148] As used herein, "pharmaceutically acceptable carrier" or "pharmaceutical acceptable excipient" includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, various types of wetting agents, detergents such as polysorbate 20 to prevent aggregation, and sugars such as sucrose as cryoprotectant. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline (PBS) or normal (0.9%) saline. Compositions comprising such carriers are formulated by well-known conventional methods (see, for example, Remington's Pharmaceutical Sciences, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990; and Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing, 2000).

[0149] The term "k_{on}", as used herein, refers to the rate constant for association of an antibody (or bioconjugate) to an antigen. Specifically, the rate constants (k_{on} and k_{off}) and equilibrium dissociation constants are measured using full-length antibodies and/or Fab antibody fragments (i.e. univalent) and IL-6.

[0150] The term "k_{off} ", as used herein, refers to the rate constant for dissociation of an antibody (or bioconjugate) from the antibody/antigen complex.

[0151] The term "K_D", as used herein, refers to the equilibrium dissociation constant of an antibody-antigen (or bioconjugate-antigen) interaction.

[0152] Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to “about X” includes description of “X.” Numeric ranges are inclusive of the numbers defining the range.

[0153] The term "patient" includes human and other subjects (including mammals) that receive either prophylactic or therapeutic treatment.

[0154] For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic side chains): met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative

substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

[0155] Percentage sequence identities are determined with antibody sequences maximally aligned by the Kabat numbering convention for a variable region or EU numbering for a constant region. After alignment, if a subject antibody region (e.g., the entire mature variable region of a heavy or light chain) is being compared with the same region of a reference antibody, the percentage sequence identity between the subject and reference antibody regions is the number of positions occupied by the same amino acid in both the subject and reference antibody region divided by the total number of aligned positions of the two regions, with gaps not counted, multiplied by 100 to convert to percentage. Sequence identities of other sequences can be determined by aligning sequences using algorithms, such as BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, WI, using default gap parameters, or by inspection, and the best alignment (*i.e.*, resulting in the highest percentage of sequence similarity over a comparison window). Percentage of sequence identity is calculated by comparing two optimally aligned sequences over a window of comparison, determining the number of positions at which the identical residues occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

[0156] The term "antibody-dependent cellular cytotoxicity", or ADCC, is a mechanism for inducing cell death that depends upon the interaction of antibody-coated target cells (i.e., cells with bound antibody) with immune cells possessing lytic activity (also referred to as effector cells). Such effector cells include natural killer cells, monocytes/macrophages and neutrophils. ADCC is triggered by interactions between the Fc region of an antibody bound to a cell and Fcγ receptors, particularly FcγRI and FcγRIII, on immune effector cells such as neutrophils, macrophages and natural killer cells. The target cell is eliminated by phagocytosis or lysis, depending on the type of mediating effector cell. Death of the antibody-coated target cell occurs as a result of effector cell activity.

[0157] A humanized antibody is a genetically engineered antibody in which the CDRs from a non-human "donor" antibody are grafted into a human "acceptor" antibody sequences (see, e.g., Queen, US 5,530,101 and 5,585,089; Winter, US 5,225,539, Carter, US 6,407,213, Adair, US

5,859,205 6,881,557, Foote, US 6,881,557). The acceptor antibody sequences can be, for example, a mature human antibody sequence, a composite of such sequences, a consensus sequence of human antibody sequences, or a germline region sequence. Thus, a humanized antibody is an antibody having some or all CDRs entirely or substantially from a donor antibody and variable region framework sequences and constant regions, if present, entirely or substantially from human antibody sequences. Similarly a humanized heavy chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody heavy chain, and a heavy chain variable region framework sequence and heavy chain constant region, if present, substantially from human heavy chain variable region framework and constant region sequences. Similarly a humanized light chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody light chain, and a light chain variable region framework sequence and light chain constant region, if present, substantially from human light chain variable region framework and constant region sequences. Other than nanobodies and dAbs, a humanized antibody comprises a humanized heavy chain and a humanized light chain. A CDR in a humanized antibody is substantially from a corresponding CDR in a non-human antibody when at least 85%, 90%, 95% or 100% of corresponding residues (as defined by Kabat) are identical between the respective CDRs. The variable region framework sequences of an antibody chain or the constant region of an antibody chain are substantially from a human variable region framework sequence or human constant region respectively when at least 85, 90, 95 or 100% of corresponding residues defined by Kabat are identical.

[0158] Although humanized antibodies often incorporate all six CDRs (preferably as defined by Kabat) from a mouse antibody, they can also be made with less than all CDRs (e.g., at least 3, 4, or 5 CDRs from a mouse antibody) (e.g., Pascalis et al, J. Immunol. 169:3076, 2002; Vajdos et al., Journal of Molecular Biology, 320: 415-428, 2002; Iwahashi et al., Mol. Immunol. 36: 1079-1091, 1999; Tamura et al, Journal of Immunology, 164: 1432-1441 , 2000).

[0159] A chimeric antibody is an antibody in which the mature variable regions of light and heavy chains of a non-human antibody (e.g., a mouse) are combined with human light and heavy chain constant regions. Such antibodies substantially or entirely retain the binding specificity of the mouse antibody and are about two-thirds human sequence.

[0160] A veneered antibody is a type of humanized antibody that retains some and usually all of the CDRs and some of the non-human variable region framework residues of a non-

human antibody but replaces other variable region framework residues that may contribute to B- or T-cell epitopes, for example exposed residues (Padlan, Mol. Immunol. 28:489, 1991) with residues from the corresponding positions of a human antibody sequence. The result is an antibody in which the CDRs are entirely or substantially from a non-human antibody and the variable region frameworks of the non-human antibody are made more human-like by the substitutions. A human antibody can be isolated from a human, or otherwise result from expression of human immunoglobulin genes (e.g., in a transgenic mouse, in vitro or by phage display). Methods for producing human antibodies include the trioma method of Oestberg et al., Hybridoma 2:361-367 (1983); Oestberg, U.S. Patent No. 4,634,664; and Engleman et al., US Patent 4,634,666, use of transgenic mice including human immunoglobulin genes (see, e.g., Lonberg et al., W093/12227 (1993); US 5,877,397, US 5,874,299, US 5,814,318, US 5,789,650, US 5,770,429, US 5,661,016, US 5,633,425, US 5,625,126, US 5,569,825, US 5,545,806, Nature 148, 1547-1553 (1994), Nature Biotechnology 14, 826 (1996), Kucherlapati, WO 91/10741 (1991) and phage display methods (see, .e.g. Dower et al., WO 91/17271 and McCafferty et al., WO 92/01047, US 5,877,218, US 5,871,907, US 5,858,657, US 5,837,242, US 5,733,743 and US 5,565,332.

[0161] A "polymer" is a molecule composed of many repeating subunits. The subunits, also sometimes referred to as "monomers" can be the same or different. There are both natural and synthetic polymers. DNA, protein and complex carbohydrates are examples of natural polymers. Poly-styrene and poly-acrylamide are examples of synthetic polymers. A polymer composed of repeating units of a single monomer is called a homopolymer. A polymer composed of two or more monomers is called a copolymer or sometimes a heteropolymer. A copolymer in which certain monomer types are clustered together are sometimes called block copolymers. Polymers can be linear or branched. When the polymer is branched, polymer chains having a common origin are sometimes referred to as a polymer arm(s).

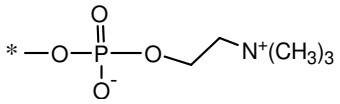
[0162] An "initiator" is a compound capable of serving as a substrate on which one or more polymerizations can take place using monomers or comonomers as described herein. The polymerization can be a conventional free radical polymerization or preferably a controlled/"living" radical polymerization, such as Atom Transfer Radical Polymerization (ATRP), Reversible Addition-Fragmentation-Termination (RAFT) polymerization or nitroxide mediated polymerization (NMP). The polymerization can be a "pseudo" controlled polymerization, such as degenerative transfer. Initiators suitable for ATRP contain one or more

labile bonds which can be homolytically cleaved to form an initiator fragment, I, being a radical capable of initiating a radical polymerization, and a radical scavenger, I', which reacts with the radical of the growing polymer chain to reversibly terminate the polymerization. The radical scavenger I' is typically a halogen, but can also be an organic moiety, such as a nitrile. In some embodiments of the present invention, the initiator contains one or more 2-bromoisobutyrate groups as sites for polymerization via ATRP.

[0163] A "chemical linker" refers to a chemical moiety that links two groups together, such as a half-life extending moiety and a protein. The linker can be cleavable or non-cleavable. Cleavable linkers can be hydrolysable, enzymatically cleavable, pH sensitive, photolabile, or disulfide linkers, among others. Other linkers include homobifunctional and heterobifunctional linkers. A "linking group" is a functional group capable of forming a covalent linkage consisting of one or more bonds to a bioactive agent. Non-limiting examples include those illustrated in Table 1 of WO2013059137 (incorporated by reference).

[0164] The term "reactive group" refers to a group that is capable of reacting with another chemical group to form a covalent bond, i.e. is covalently reactive under suitable reaction conditions, and generally represents a point of attachment for another substance. The reactive group is a moiety, such as maleimide or succinimidyl ester, is capable of chemically reacting with a functional group on a different moiety to form a covalent linkage. Reactive groups generally include nucleophiles, electrophiles and photoactivatable groups.

[0165] As used herein, "phosphorylcholine," also denoted as "PC," refers to the following:



[0166] where * denotes the point of attachment. The phosphorylcholine is a zwitterionic group and includes salts (such as inner salts), and protonated and deprotonated forms thereof.

[0167] As used herein, "phosphorylcholine-based polymer" is a polymer that contains phosphorylcholine. "Zwitterion containing polymer" refers to a polymer that contains a zwitterion.

[0168] Poly(acryloyloxyethyl phosphorylcholine) containing polymer refers to a polymer containing 2-(acryloyloxy)ethyl-2-(trimethylammonium)ethyl phosphate as monomer.

[0169] Poly(methacryloyloxyethyl phosphorylcholine) containing polymer refers to a polymer containing 2-(methacryloyloxy)ethyl-2-(trimethylammonium)ethyl phosphate as monomer.

[0170] As used herein, "molecular weight" in the context of the polymer can be expressed as either a number average molecular weight, or a weight average molecular weight or a peak molecular weight. Unless otherwise indicated, all references to molecular weight herein refer to the peak molecular weight. These molecular weight determinations, number average (Mn), weight average (Mw) and peak (Mp), can be measured using size exclusion chromatography or other liquid chromatography techniques. Other methods for measuring molecular weight values can also be used, such as the use of end-group analysis or the measurement of colligative properties (*e.g.*, freezing-point depression, boiling-point elevation, or osmotic pressure) to determine number average molecular weight, or the use of light scattering techniques, ultracentrifugation or viscometry to determine weight average molecular weight. In a preferred embodiment of the present invention, the molecular weight is measured by SEC-MALS (size exclusion chromatography – multi angle light scattering). The polymeric reagents of the invention are typically polydisperse (*i.e.*, number average molecular weight and weight average molecular weight of the polymers are not equal). The Poly Dispersity Index (PDI) provides a measure for the dispersity of polymers in a mixture. PDI is given by the formula Mw/Mn. In this regard a homogenous protein will have a PDI of 1.0 (Mn is the same as Mw). Typically, the PDI for polymers will be above 1.0. Polymers in accordance with the present invention preferably have relatively low polydispersity (PDI) values of, for example, less than about 1.5, as judged, for example, by SEC-MALS. In other embodiments, the polydispersities (PDI) are more preferably in the range of about 1.4 to about 1.2, still more preferably less than about 1.15, and still more preferably less than about 1.10, yet still more preferably less than about 1.05, and most preferably less than about 1.03.

[0171] As used herein, "protected," "protected form," "protecting group" and "protective group" refer to the presence of a group (*i.e.*, the protecting group) that prevents or blocks reaction of a particular chemically reactive functional group in a molecule under certain reaction conditions. Protecting groups vary depending upon the type of chemically reactive group being protected as well as the reaction conditions to be employed and the presence of additional reactive or protecting groups in the molecule, if any. Suitable protecting groups include those such

as found in the treatise by Greene et al., "Protective Groups In Organic Synthesis," 3rd Edition, John Wiley and Sons, Inc., New York, 1999.

[0172] As used herein, "alkyl" refers to a straight or branched, saturated, aliphatic radical having the number of carbon atoms indicated. For example, C_1-C_6 alkyl includes, but is not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, hexyl, etc. Other alkyl groups include, but are not limited to heptyl, octyl, nonyl, decyl, etc. Alkyl can include any number of carbons, such as 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 2-3, 2-4, 2-5, 2-6, 3-4, 3-5, 3-6, 4-5, 4-6 and 5-6 carbons.

[0173] The term "lower" referred to above and hereinafter in connection with organic radicals or compounds respectively defines a compound or radical which can be branched or unbranched with up to and including 7, preferably up to and including 4 and (as unbranched) one or two carbon atoms.

[0174] As used herein, "alkylene" refers to an alkyl group, as defined above, linking at least two other groups, *i.e.*, a divalent hydrocarbon radical. The two moieties linked to the alkylene can be linked to the same atom or different atoms of the alkylene. For instance, a straight chain alkylene can be the bivalent radical of $-(CH_2)_n$, where n is 1, 2, 3, 4, 5 or 6. Alkylene groups include, but are not limited to, methylene, ethylene, propylene, isopropylene, butylene, isobutylene, sec-butylene, pentylene and hexylene.

[0175] Substituents for the alkyl, alkenyl, alkylene, heteroalkyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl radicals can be one or more of a variety of groups selected from, but not limited to: -OR', =O, =NR',
=N-OR', -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -C(O)R', $-CO_2R'$, -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R"', $-NR"C(O)_2R'$, -NR-C(NR'R"R"')=NR"", -NR-C(NR'R")=NR"', $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2NR'R"$, $-NRSO_2R'$, -CN and $-NO_2$ in a number ranging from 1 to (2m'+1), where m' is the total number of carbon atoms in such radical. Each of R', R", R"' and R"" independently refers to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, *e.g.*, aryl substituted with 1-3 halogens, substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. When R' and R" are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R" is meant to include, but not be limited to, 1-pyrrolidinyl and 4-morpholinyl.

[0176] As used herein, "alkoxy" refers to alkyl group attached to an oxygen atom and forms radical –O-R, wherein R is alkyl. Alkoxy groups include, for example, methoxy, ethoxy, propoxy, iso-propoxy, butoxy, 2-butoxy, iso-butoxy, sec-butoxy, tert-butoxy, pentoxy, hexoxy, etc. The alkoxy groups can be further substituted with a variety of substituents described herein. For example, the alkoxy groups can be substituted with halogens to form a "halo-alkoxy" group.

[0177] As used herein, "carboxyalkyl" means an alkyl group (as defined herein) substituted with a carboxy group. The term "carboxycycloalkyl" means an cycloalkyl group (as defined herein) substituted with a carboxy group. The term alkoxyalkyl means an alkyl group (as defined herein) substituted with an alkoxy group. The term "carboxy" employed herein refers to carboxylic acids and their esters.

[0178] As used herein, "haloalkyl" refers to alkyl as defined above where some or all of the hydrogen atoms are substituted with halogen atoms. Halogen (halo) preferably represents chloro or fluoro, but may also be bromo or iodo. For example, haloalkyl includes trifluoromethyl, fluoromethyl, 1,2,3,4,5-pentafluoro-phenyl, etc. The term "perfluoro" defines a compound or radical which has all available hydrogens that are replaced with fluorine. For example, perfluorophenyl refers to 1,2,3,4,5-pentafluorophenyl, perfluoromethyl refers to 1,1,1-trifluoromethyl, and perfluoromethoxy refers to 1,1,1-trifluoromethoxy. Haloalkyl can also be referred to as halo-substitute alkyl, such as fluoro-substituted alkyl.

[0179] As used herein, "cytokine" in the context of this invention is a member of a group of protein signaling molecules that may participate in cell-cell communication in immune and inflammatory responses. Cytokines are typically small, water-soluble glycoproteins that have a mass of about 8-35 kDa.

[0180] As used herein, "cycloalkyl" refers to a saturated mono- or multi- cyclic aliphatic ring system that contains from about 3 to 12, from 3 to 10, from 3 to 7, or from 3 to 6 carbon atoms. When cycloalkyl group is composed of two or more rings, the rings may be joined together with a fused ring or a spiro ring structure. When cycloalkyl group is composed of three or more rings, the rings may also join together forming a bridged ring structure. Monocyclic rings include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cyclooctyl. Bicyclic and polycyclic rings include, for example, bicyclo[1.1.1]pentane, bicyclco[2.1.1]heptane, norbornane, decahydronaphthalene and adamantane. For example, C_{3-8} cycloalkyl includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclooctyl, and norbornane.

[0181] As used herein, "endocyclic" refers to an atom or group of atoms which comprise part of a cyclic ring structure.

[0182] As used herein, "exocyclic" refers to an atom or group of atoms which are attached but do not define the cyclic ring structure.

[0183] As used herein, "cyclic alkyl ether" refers to a 4 or 5 member cyclic alkyl group having 3 or 4 endocyclic carbon atoms and 1 endocyclic oxygen or sulfur atom (*e.g.*, oxetane, thietane, tetrahydrofuran, tetrahydrothiophene); or a 6 to 7 member cyclic alkyl group having 1 or 2 endocyclic oxygen or sulfur atoms (*e.g.*, tetrahydropyran, 1,3-dioxane, 1,4-dioxane, tetrahydrothiopyran, 1,3-dithiane, 1,4-dithiane, 1,4-oxathiane).

[0184] As used herein, "alkenyl" refers to either a straight chain or branched hydrocarbon of 2 to 6 carbon atoms, having at least one double bond. Examples of alkenyl groups include, but are not limited to, vinyl, propenyl, isopropenyl, 1-butenyl, 2-butenyl, isobutenyl, butadienyl, 1-pentenyl, 2-pentenyl, isopentenyl, 1,3-pentadienyl, 1,4-pentadienyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,5-hexadienyl, 2,4-hexadienyl, or 1,3,5-hexatrienyl. Alkenyl groups can also have from 2 to 3, 2 to 4, 2 to 5, 3 to 4, 3 to 5, 3 to 6, 4 to 5, 4 to 6 and 5 to 6 carbons.

[0185] As used herein, "alkenylene" refers to an alkenyl group, as defined above, linking at least two other groups, *i.e.*, a divalent hydrocarbon radical. The two moieties linked to the alkenylene can be linked to the same atom or different atoms of the alkenylene. Alkenylene groups include, but are not limited to, ethenylene, propenylene, isopropenylene, butenylene, isobutenylene, sec-butenylene, pentenylene and hexenylene.

[0186] As used herein, "alkynyl" refers to either a straight chain or branched hydrocarbon of 2 to 6 carbon atoms, having at least one triple bond. Examples of alkynyl groups include, but are not limited to, acetylenyl, propynyl, 1-butynyl, 2-butynyl, isobutynyl, sec-butynyl, butadiynyl, 1-pentynyl, 2-pentynyl, isopentynyl, 1,3-pentadiynyl, 1,4-pentadiynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 1,3-hexadiynyl, 1,4-hexadiynyl, 1,5-hexadiynyl, 2,4-hexadiynyl, or 1,3,5-hexatriynyl. Alkynyl groups can also have from 2 to 3, 2 to 4, 2 to 5, 3 to 4, 3 to 5, 3 to 6, 4 to 5, 4 to 6 and 5 to 6 carbons.

[0187] As used herein, "alkynylene" refers to an alkynyl group, as defined above, linking at least two other groups, *i.e.*, a divalent hydrocarbon radical. The two moieties linked to the alkynylene can be linked to the same atom or different atoms of the alkynylene. Alkynylene

groups include, but are not limited to, ethynylene, propynylene, butynylene, sec-butynylene, pentynylene and hexynylene.

[0188] As used herein, "cycloalkylene" refers to a cycloalkyl group, as defined above, linking at least two other groups, *i.e.*, a divalent hydrocarbon radical. The two moieties linked to the cycloalkylene can be linked to the same atom or different atoms of the cycloalkylene. Cycloalkylene groups include, but are not limited to, cyclopropylene, cyclobutylene, cyclopentylene, cyclohexylene, and cyclooctylene.

[0189] As used herein, "heterocycloalkyl" refers to a ring system having from 3 ring members to about 20 ring members and from 1 to about 5 heteroatoms such as N, O and S. Additional heteroatoms can also be useful, including, but not limited to, B, Al, Si and P. The heteroatoms can also be oxidized, such as, but not limited to, -S(O)- and $-S(O)_2-$. For example, heterocycle includes, but is not limited to, tetrahydrofuranyl, tetrahydrothiophenyl, morpholino, pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, piperidinyl, indolinyl, quinuclidinyl and 1,4-dioxa-8-aza-spiro[4.5]dec-8-yl.

[0190] As used herein, "heterocycloalkylene" refers to a heterocyclalkyl group, as defined above, linking at least two other groups. The two moieties linked to the heterocycloalkylene can be linked to the same atom or different atoms of the heterocycloalkylene.

[0191] As used herein, "aryl" refers to a monocyclic or multicyclic (e.g., fused bicyclic, tricyclic or greater) aromatic ring assembly containing 6 to 16 carbon atoms. For example, aryl may be phenyl, benzyl or naphthyl, preferably phenyl. Aryl groups can be mono-, di- or tri-substituted by one, two or three radicals selected from alkyl, alkoxy, aryl, hydroxy, halogen, cyano, amino, amino-alkyl, trifluoromethyl, alkylenedioxy and oxy-C_2-C_3-alkylene; all of which are optionally further substituted, for instance as hereinbefore defined; or 1- or 2-naphthyl; or 1- or 2-phenanthrenyl. Alkylenedioxy is a divalent substitute attached to two adjacent carbon atoms of phenyl, e.g. methylenedioxy or ethylenedioxy. Oxy-C_2-C_3-alkylene is also a divalent substituent attached to two adjacent carbon atoms of phenyl, e.g. oxyethylene or oxypropylene. An example for oxy- C_2-C_3-alkylene-phenyl is 2,3-dihydrobenzofuran-5-yl.

[0192] Preferred as aryl is naphthyl, phenyl or phenyl mono- or disubstituted by alkoxy, phenyl, halogen, alkyl or trifluoromethyl, especially phenyl or phenyl-mono- or disubstituted by alkoxy, halogen or trifluoromethyl, and in particular phenyl.

[0193] Examples of substituted phenyl groups as R are, *e.g.* 4-chlorophen-1-yl, 3,4-dichlorophen-1-yl, 4-methoxyphen-1-yl, 4-methylphen-1-yl, 4-aminomethylphen-1-yl, 4-methoxyethylaminomethylphen-1-yl, 4-hydroxyethylaminomethylphen-1-yl, 4-hydroxyethyl-(methyl)-aminomethylphen-1-yl, 3-aminomethylphen-1-yl, 4-N-acetylaminomethylphen-1-yl, 4-aminophen-1-yl, 3-aminophen-1-yl, 2-aminophen-1-yl, 4-phenyl-phen-1-yl, 4-(imidazol-1-yl)-phenyl, 4-(imidazol-1-ylmethyl)-phen-1-yl, 4-(morpholin-1-yl)-phen-1-yl, 4-(morpholin-1-ylmethyl)-phen-1-yl, 4-(2-methoxyethylaminomethyl)-phen-1-yl and 4-(pyrrolidin-1-ylmethyl)-phen-1-yl, 4-(thiophenyl)-phen-1-yl, 4-(3-thiophenyl)-phen-1-yl, 4-(4-methylpiperazin-1-yl)-phen-1-yl, and 4-(piperidinyl)-phenyl and 4-(pyridinyl)-phenyl optionally substituted in the heterocyclic ring.

[0194] As used herein, "arylene" refers to an aryl group, as defined above, linking at least two other groups. The two moieties linked to the arylene are linked to different atoms of the arylene. Arylene groups include, but are not limited to, phenylene.

[0195] As used herein, "arylene-oxy" refers to an arylene group, as defined above, where one of the moieties linked to the arylene is linked through an oxygen atom. Arylene-oxy groups include, but are not limited to, phenylene-oxy.

[0196] Similarly, substituents for the aryl and heteroaryl groups are varied and are selected
from: -halogen, -OR', -OC(O)R', -NR'R", -SR', -R', -CN, $-NO_2$, $-CO_2R'$, -CONR'R", -C(O)R', -OC(O)NR'R", -NR"C(O)R', $-NR"C(O)_2R'$, -NR'-C(O)NR"R"', $-NH-C(NH_2)=NH$, $-NR'C(NH_2)=NH$, $-NH-C(NH_2)=NR'$, -S(O)R', $-S(O)_2R'$, $-S(O)_2NR'R"$, $-N_3$, $-CH(Ph)_2$, perfluoro(C_1-C_4)alkoxy, and perfluoro(C_1-C_4)alkyl, in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R" and R"' are independently selected from hydrogen, (C_1-C_8)alkyl and heteroalkyl, unsubstituted aryl and heteroaryl, (unsubstituted aryl)-(C_1-C_4)alkyl, and (unsubstituted aryl)oxy-(C_1-C_4)alkyl.

[0197] Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-T-C(O)-(CH_2)_q-U-$, wherein T and U are independently -NH-, -O-, $-CH_2-$ or a single bond, and q is an integer of from 0 to 2. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-A-(CH_2)_r-B-$, wherein A and B are independently $-CH_2-$, -O-, -NH-, -S-, -S(O)-, $-S(O)_2-$, $-S(O)_2NR'-$ or a single bond, and r is an

integer of from 1 to 3. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-(CH_2)_s-X-(CH_2)_t-$, where s and t are independently integers of from 0 to 3, and X is -O-, -NR'-, -S-, -S(O)-, $-S(O)_2-$, or $-S(O)_2NR'-$. The substituent R' in -NR'- and $-S(O)_2NR'-$ is selected from hydrogen or unsubstituted (C_1-C_6)alkyl.

[0198] As used herein, "heteroaryl" refers to a monocyclic or fused bicyclic or tricyclic aromatic ring assembly containing 5 to 16 ring atoms, where from 1 to 4 of the ring atoms are a heteroatom each N, O or S. For example, heteroaryl includes pyridyl, indolyl, indazolyl, quinoxalinyl, quinolinyl, isoquinolinyl, benzothienyl, benzofuranyl, furanyl, pyrrolyl, thiazolyl, benzothiazolyl, oxazolyl, isoxazolyl, triazolyl, tetrazolyl, pyrazolyl, imidazolyl, thienyl, or any other radicals substituted, especially mono- or di-substituted, by e.g. alkyl, nitro or halogen. Pyridyl represents 2-, 3- or 4-pyridyl, advantageously 2- or 3-pyridyl. Thienyl represents 2- or 3-thienyl. Quinolinyl represents preferably 2-, 3- or 4-quinolinyl. Isoquinolinyl represents preferably 1-, 3- or 4-isoquinolinyl. Benzopyranyl, benzothiopyranyl represents preferably 3-benzopyranyl or 3-benzothiopyranyl, respectively. Thiazolyl represents preferably 2- or 4-thiazolyl, and most preferred, 4-thiazolyl. Triazolyl is preferably 1-, 2- or 5-(1,2,4-triazolyl). Tetrazolyl is preferably 5-tetrazolyl.

[0199] Preferably, heteroaryl is pyridyl, indolyl, quinolinyl, pyrrolyl, thiazolyl, isoxazolyl, triazolyl, tetrazolyl, pyrazolyl, imidazolyl, thienyl, furanyl, benzothiazolyl, benzofuranyl, isoquinolinyl, benzothienyl, oxazolyl, indazolyl, or any of the radicals substituted, especially mono- or di-substituted.

[0200] The term "heteroalkyl" refers to an alkyl group having from 1 to 3 heteroatoms such as N, O and S. Additional heteroatoms can also be useful, including, but not limited to, B, Al, Si and P. The heteroatoms can also be oxidized, such as, but not limited to, -S(O)- and $-S(O)_2-$. For example, heteroalkyl can include ethers, thioethers, alkyl-amines and alkyl-thiols.

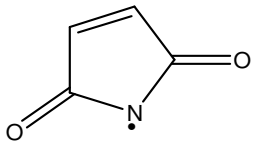
[0201] The term "heteroalkylene" refers to a heteroalkyl group, as defined above, linking at least two other groups. The two moieties linked to the heteroalkylene can be linked to the same atom or different atoms of the heteroalkylene.

[0202] As used herein, "electrophile" refers to an ion or atom or collection of atoms, which may be ionic, having an electrophilic center, *i.e.*, a center that is electron seeking, capable

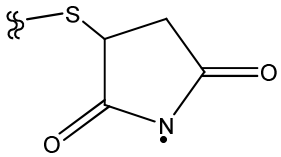
of reacting with a nucleophile. An electrophile (or electrophilic reagent) is a reagent that forms a bond to its reaction partner (the nucleophile) by accepting both bonding electrons from that reaction partner.

[0203] As used herein, "nucleophile" refers to an ion or atom or collection of atoms, which may be ionic, having a nucleophilic center, *i.e.*, a center that is seeking an electrophilic center or capable of reacting with an electrophile. A nucleophile (or nucleophilic reagent) is a reagent that forms a bond to its reaction partner (the electrophile) by donating both bonding electrons. A "nucleophilic group" refers to a nucleophile after it has reacted with a reactive group. Non limiting examples include amino, hydroxyl, alkoxy, haloalkoxy and the like.

[0204] As used herein, "maleimido" refers to a pyrrole-2,5-dione-1-yl group having the structure:



[0205] which upon reaction with a sulfhydryl (*e.g.*, a thio alkyl) forms an -S-maleimido group having the structure



[0206] where "•" indicates the point of attachment for the maleimido group and "§ "indicates the point of attachment of the sulfur atom the thiol to the remainder of the original sulfhydryl bearing group.

[0207] For the purpose of this disclosure, "naturally occurring amino acids" found in proteins and polypeptides are L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamine, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, and or L-valine. "Non-naturally occurring amino acids" found in proteins are any amino acid other than those recited as naturally occurring amino acids. Non-naturally occurring amino acids include, without limitation, the D isomers of the naturally occurring amino acids, and mixtures of D and L isomers of the naturally occurring amino acids. Other amino acids, such as 4-hydroxyproline, desmosine, isodesmosine, 5-hydroxylysine, epsilon-N-methyllysine, 3-methylhistidine, although

found in naturally occurring proteins, are considered to be non-naturally occurring amino acids found in proteins for the purpose of this disclosure as they are generally introduced by means other than ribosomal translation of mRNA.

[0208] As used herein, "linear" in reference to the geometry, architecture or overall structure of a polymer, refers to polymer having a single polymer arm.

[0209] As used herein, "branched," in reference to the geometry, architecture or overall structure of a polymer, refers to a polymer having 2 or more polymer "arms" extending from a core structure contained within an initiator. The initiator may be employed in an atom transfer radical polymerization (ATRP) reaction. A branched polymer may possess 2 polymer chains (arms), 3 polymer arms, 4 polymer arms, 5 polymer arms, 6 polymer arms, 7 polymer arms, 8 polymer arms, 9 polymer arms or more. Each polymer arm extends from a polymer initiation site. Each polymer initiation site is capable of being a site for the growth of a polymer chain by the addition of monomers. For example and not by way of limitation, using ATRP, the site of polymer initiation on an initiator is typically an organic halide undergoing a reversible redox process catalyzed by a transition metal compound such as cuprous halide. Preferably, the halide is a bromine.

[0210] As used herein, "pharmaceutically acceptable excipient" refer to an excipient that can be included in the compositions of the invention and that causes no significant adverse toxicological effect on the patient and is approved or approvable by the FDA for therapeutic use, particularly in humans. Non-limiting examples of pharmaceutically acceptable excipients include water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose and the like.

[0211] As used herein, "OG1786" is a 9-arm initiator used for polymer synthesis with the structure shown in FIG. 2D, which depicts that salt form of OG1786 with trifluororacetic acid. OG1786 may be used in accordance with the present invention as other salts or as the free base.

[0212] As used herein, "OG1801" is an approximately (+/- 15%) 750 kDa polymer (either by Mn or Mp) made using OG1786 as an initiator for ATRP synthesis using the monomer HEMA-PC. The structure of OG1801 is shown in FIG. 2J.

[0213] As used herein, "OG1802" is OG1801 with a maleimide functionality added, and it has the structure shown in FIG. 2K, wherein each of n_1, n_2, n_3, n_4, n_5, n_6, n_7, n_8 and n_9 is an integer (positive) (from 0 up to about 3000) such that the total molecular weight of the polymer

is (Mw) 750,000 ± 15% Daltons. When the term OG1802 is used to modify a protein term (such as VEGF trap or anti-IL-6 antibody), it designates that the protein is the conjugate protein.

[0214] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present specification, including definitions, will control. Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Any example(s) following the term “e.g.” or “for example” is not meant to be exhaustive or limiting.

[0215] The phrase “a” or “an” entity refers to one or more of that entity; for example, a compound refers to one or more compounds or at least one compound. As such, the terms “a” (or “an”), “one or more”, and “at least one” can be used interchangeably herein.

[0216] As used herein, “about” means variation one might see in measurements taken among different instruments, samples, and sample preparations.

[0217] As used herein, CDR positions follow their order of appearance in variable domain when described. For example, heavy chain positions can be described as S35H or G66D. As used herein, Fc positions follow EU numbering when described or when noted to be in EU numbering. For example, mutations of antibodies can be described as L234A or L235A, which would be according to EU numbering. Positions may also be defined according to specified positions within a specific SEQ ID or sequence provided herein.

[0218] Multi-angle light scattering (**MALS**) is a technique of analyzing macromolecules where the laser light impinges on the molecule, the oscillating electric field of the light induces an oscillating dipole within it. This oscillating dipole will re-radiate light and can be measured using a MALS detector such as Wyatt miniDawn TREOS. The intensity of the radiated light depends on the magnitude of the dipole induced in the macromolecule which in turn is proportional to the polarizability of the macromolecule, the larger the induced dipole, and hence, the greater the intensity of the scattered light. Therefore, in order to analyze the scattering from a solution of such macromolecules, one should know their polarizability relative to the surrounding medium (e.g., the solvent). This may be determined from a measurement of the change, Δn, of the solution's refractive index n with the molecular concentration change, Δc, by measuring

the ***dn/dc*** (=$\Delta n/\Delta c$) value using a Wyatt Optilab T-rEX differential refractometer. Two molar weight parameters that MALS determination employ are number average molecular weight (Mn) and weight average molecular weight (Mw) where the polydispersity index (PDI) equals Mw divided by Mn. SEC also allows another average molecular weight determination of the peak molecular weight Mp which is defined as the molecular weight of the highest peak at the SEC.

[0219] The PDI is used as a measure of the broadness of a molecular weight distribution of a polymer and bioconjugate which is derived from conjugation of a discrete protein to a polydisperse biopolymer (e.g., OG1802). For a protein sample, its polydispersity is close to 1.0 due to the fact that it is a product of translation where every protein molecule in a solution is expected to have almost the same length and molar mass. In contrast, due to the polydisperse nature of the biopolymer where the various length of polymer chains are synthesized during the polymerization process, it is very important to determine the PDI of the sample as one of its quality attribute for narrow distribution of molecular weight.

[0220] Size exclusion chromatography (**SEC**) is a chromatography technique in which molecules in solution are separated by their size. Typically an aqueous solution is applied to transport the sample through the column which is packed with resins of various pore sizes. The resin is expected to be inert to the analyte when passing through the column and the analytes separate from each other based on their unique size and the pore size characteristics of the selected column.

[0221] Coupling the **SEC** with **MALS** or **SEC/MALS** provides accurate distribution of molar mass and size (root mean square radius) as opposed to relying on a set of SEC calibration standards. This type of arrangement has many advantages over traditional column calibration methods. Since the light scattering and concentration are measured for each eluting fraction, the molar mass and size can be determined independently of the elution position. This is particularly relevant for species with non-globular shaped macromolecules such as the biopolymers (OG1802) or bioconjugates; such species typically do not elute in a manner that might be described by a set of column calibration standards.

[0222] In some embodiments, a SEC/MALS analysis includes a Waters HPLC system with Alliance 2695 solvent delivery module and Waters 2996 Photodiole Array Detector equipped with a Shodex SEC-HPLC column (7.8x300mm). This is connected online with a Wyatt miniDawn TREOS and Wyatt Optilab T-rEX differential refractometer. The Empower software

from Waters can be used to control the Waters HPLC system and the ASTRA V 6.1.7.16 software from Wyatt can be used to acquire the MALS data from the Wyatt miniDawn TREOS, dn/dc data from the T-rEX detector and the mass recovery data using the A280 absorbance signal from the Waters 2996 Photodiole Array detector. SEC can be carried out at 1ml/min in 1xPBS pH 7.4, upon sample injection, the MALS and RI signals can be analyzed by the ASTRA software for determination of absolute molar mass (Mp, **Mw, Mn**) and polydisperse index (**PDI**). In addition, the calculation also involves the input dn/dc values for polymer and protein as 0.142 and 0.183, respectively. For bioconjugates dn/dc value, the dn/dc is calculated based on the weighted MW of the polymer and the protein to be about 0.148 using the formula below:

$$\text{Conjugate } \mathbf{dn/dc} = 0.142 \times [\, MW_{polymer} / (MW_{polymer} + MW_{protein})] + 0.183 \times [MW_{protein} / (MW_{polymer} + MW_{protein})]$$

[0223] Where MW*polyme.r* for OG1802 measured by SEC-MALS is about 800 kDa and the MW*protein* for anti-IL-6 measured by SEC-MALS is about 145 kDa, the expected total molecular weight of the bioconjugate measured by SEC-MALS is about 1000 kDa. The MW*protein* for the antiIL-6 VEGF Trap or VEGF Trap-antiIL-6 is about 192 kDa, and the expected total molecular weight of the bioconjugate is 1000-1100 kDa.

[0224] Exemplary methods and materials are described herein, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. The materials, methods, and examples are illustrative only and not intended to be limiting.

I. IL-6 ANTAGONIST ANTIBODIES, IL-6 AB-VEGF TRAPS AND/OR CONJUGATES THEREOF

[0225] Provided herein are anti-IL-6 antibodies that block, suppress or reduce (including significantly reduces) IL-6 biological activity, including downstream events mediated by IL-6. In some embodiments, the IL-6 antagonist antibody will have one or more of the CDR sequences provided herein.

[0226] In some embodiments, the isolated antagonist antibody specifically binds to IL-6.

[0227] In some embodiments, the antibody preferably reacts with IL-6 in a manner that inhibits IL-6 signaling function. In some embodiments, the IL-6 antagonist antibody specifically binds primate IL-6.

[0228] The antibodies useful in the present invention can encompass monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', $F(ab')_2$, Fv, Fc, etc.), chimeric antibodies, bispecific antibodies, heteroconjugate antibodies, single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion (e.g., a domain antibody), humanized antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. The antibodies may be murine, rat, human, or any other origin (including chimeric or humanized antibodies). In some embodiments, the IL-6 antagonist antibody is a monoclonal antibody. In some embodiments, the antibody is a human or humanized antibody.

[0229] In some embodiments, the antibody comprises a heavy chain amino acid variable region as shown in Tables 1A, 1B, 0.1 and/or 0.2 or FIG. 5. In some embodiments, an isolated antagonist antibody comprises a heavy chain variable region (VH) comprising a VH complementarity determining region one (CDR1), VH CDR2, and VH CDR3 of the VH having an amino acid sequence of that shown in Tables 1A, 1B, 0.1 and/or 0.2, and a light chain variable region (VL) comprising a VL CDR1, VL CDR2, and VL CDR3 of the VL having an amino acid sequence of that shown in the table.

[0230] In some embodiments, an isolated antagonist anti-IL-6 antibody is provided. The antibody comprises a heavy chain constant domain comprising one or more mutations to reduce effector function. In some embodiments, the one or more mutations reduce effector functions of the antibody related to the complement cascade, for example, a reduced activation of the complement cascade. In some embodiments, the reduction in effector function is at least about 50%.

[0231] In some embodiments, an isolated antagonist antibody that specifically binds to IL-6 comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein the antibody comprises the mutations L234A, L235A, and G237A (based on EU numbering) is provided. In some embodiments, the isolated antagonist antibody comprises the mutations L234A, L235A, and G237A. In some embodiments, the isolated antagonist antibody

with mutations has minimized binding to FC gamma receptors or C1q. In some embodiments, the isolated antagonist antibody with mutations L234A, L235A, and G237A has minimized binding to FC gamma receptors or C1q. In some embodiments, an isolated antagonist anti-IL-6 antibody is provided, wherein the mutation(s) is located at one or more of the following amino acid positions (EU numbering): E233, L234, L235, G236, G237, A327, A330, and P331. In some embodiments, an isolated antagonist anti-IL-6 antibody is provided, wherein the mutation (s) is selected from the group consisting of E233P, L234V, L234A, L235A, G237A, A327G, A330S, and P331S.

[0232] In some embodiments, an isolated antagonist anti-IL-6 antibody is provided. The heavy chain constant domain further comprises a cysteine residue introduced by recombinant DNA technology. In some embodiments, the cysteine residue is selected from the group consisting of Q347C and L443C (EU numbering). In some embodiments, the cysteine residue is L443C (EU numbering).

[0233] In some embodiments, the antibody comprises all three of the following mutations (EU numbering) L234A, L235A, and G237A, and the antibody comprises L443C (EU numbering). In some embodiments, the antibody is a human IgG1, and a heavy chain constant domain of the antibody comprises one or more mutations that reduce an immune-mediated effector function.

[0234] In some embodiments, an isolated antagonist antibody is provided that binds an epitope on human IL-6 that is the same as or overlaps with the epitope recognized by an antibody comprising the amino acid sequences in any one or more of Tables: 1A and 1B, and/or 11.3. In some embodiments, an IL-6 antibody with a cys and that is linked through that cysteine to a polymer is provided (as shown in Formula 17, herein).

[0235] In some embodiments, an isolated antagonist antibody that binds to IL-6 is provided. In some embodiments, the isolated antagonist antibody that binds to IL-6 comprises a heavy chain comprising the amino acid sequence shown in Tables 1A, 1B, 0.1 and/or 0.2, with or without the C-terminal lysine and a light chain comprising the amino acid sequence shown in Tables 1A, 1B, 0.1 and/or 0.2.

[0236] In some embodiments, an isolated antagonist antibody that binds to IL-6 is provided. The antibody comprises a VH comprising the amino acid sequence shown in Tables 1A, 1B, 0.1 and/or 0.2, or a sequence that is at least 90% identical thereto, having amino acid substitutions in residues that are not within a CDR. In some embodiments, the antibody comprises

one or more of: HCDR1: in FIG. 5, HCDR2: in FIG. 5, HCDR3: in FIG. 5; LCDR1: in FIG. 5, LCDR2: in FIG. 5, LCDR3: in FIG. 5, for example, 1, 2, 3, 4, 5, or all 6 CDRs.

[0237] In some embodiments, an antibody that binds to IL-6 is provided, wherein the antibody comprises a CDRH1 that is the CDRH1 in Tables 1A, 1B, 0.1 and/or 0.2, a CDRH2 that is the CDRH2 in Tables 1A, 1B, 0.1 and/or 0.2, a CDRH3 that is the CDRH3 in Tables 1A, 1B, 0.1 and/or 0.2, a CDRL1 that is the CDRL1 in Tables 1A, 1B, 0.1 and/or 0.2, a CDRL2 that is the CDRL2 in Tables 1A, 1B, 0.1 and/or 0.2, a CDRL3 that is the CDRL3 in Tables 1A, 1B, 0.1 and/or 0.2, at least one of the following mutations: L234A, L235A, and G237A based on EU numbering, and at least one of the following mutations:Q347C or L443C based on EU numbering.

[0238] In some embodiments, an isolated antagonist anti-IL-6 antibody is provided. The heavy chain variable region of the antibody comprises three complementarity determining regions (CDRs) comprising the amino acid sequences shown in Table 1A. In some embodiments, an isolated antagonist anti-IL-6 antibody is provided, wherein the light chain variable region of the antibody comprises three complementarity determining regions (CDRs) comprising the amino acid sequences shown in Table 1B. In some embodiments, the antibody is one that contains one or more of the identified sequences in FIG. 5, e.g., one or more of the CDRS (including 2, 3, 4, 5 or 6 of the boxed CDRs) and/or the entire heavy and light chain variable regions.

[0239] In some embodiments, an isolated antagonist anti-IL-6 antibody comprises a heavy chain variable region (VH) that comprises three CDRs comprising the amino acid sequences shown in Table 1A, and the light chain variable region (VL) of the antibody comprises three CDRs comprising the amino acid sequences shown in Table 1B.

[0240] In some embodiments, an isolated antagonist anti-IL-6 antibody is provided. The VH comprises the amino acid sequences shown in Table 1A and the light chain variable region of the antibody comprises three CDRs comprising the amino acid sequences shown in Table 1B.

[0241] In some embodiments, an isolated antagonist anti-IL-6 antibody is provided, wherein the antibody comprises a VL comprising the amino acid sequence shown in Table 1B, or a variant thereof with one amino acid substitution in amino acids that are not within a CDR. In some embodiments, an isolated antagonist anti-IL-6 antibody is provided, wherein the antibody comprises a VH comprising the amino acid sequence shown in Table 1A, or a variant thereof with several amino acid substitutions in amino acids that are not within a CDR.

[0242] In some embodiments, an isolated antagonist antibody is provided, wherein the antibody comprises a heavy chain comprising the amino acid sequence shown in Table 1A, with a C-terminal lysine, and a light chain comprising the amino acid sequence shown in Table 1B. In some embodiments, an isolated antagonist antibody is provided, wherein the antibody comprises a heavy chain comprising the amino acid sequence shown in Table 1A, without a C-terminal lysine, and a light chain comprising the amino acid sequence shown in Table 1B.

[0243] The IL-6 antagonist antibodies may be made by any method known in the art. General techniques for production of human and mouse antibodies are known in the art and/or are described herein.

[0244] IL-6 antagonist antibodies can be identified or characterized using methods known in the art, whereby reduction, amelioration, or neutralization of IL-6 biological activity is detected and/or measured. In some embodiments, an IL-6 antagonist antibody is identified by incubating a candidate antibody with IL-6 and monitoring binding to IL-6R or IL-6/IL-6R binding to gp130 and/or attendant reduction or neutralization of a biological activity of IL-6. The binding assay may be performed with, e.g., purified IL-6 polypeptide(s), or with cells naturally expressing (e.g., various strains), or transfected to express, IL-6 polypeptide(s). In one embodiment, the binding assay is a competitive binding assay, where the ability of a candidate antibody to compete with a known IL-6 antagonist antibody for IL-6 binding is evaluated. The assay may be performed in various formats, including the ELISA format.

[0245] Following initial identification, the activity of a candidate IL-6 antagonist antibody can be further confirmed and refined by bioassays, known to test the targeted biological activities. In some embodiments, an in vitro cell assay is used to further characterize a candidate IL-6 antagonist antibody.

[0246] IL-6 antagonist antibodies may be characterized using methods well known in the art. For example, one method is to identify the epitope to which it binds, or "epitope mapping." There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, Using Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999. In an additional example, epitope mapping can be used to determine the sequence to which an IL-6 antagonist antibody binds. IL-6

antagonist antibody Epitope mapping is commercially available from various sources, for example, Pepscan Systems (Edelhertweg 15, 8219 PH Lelystad, The Netherlands). The epitope can be a linear epitope, i.e., contained in a single stretch of amino acids, or a conformational epitope formed by a three-dimensional interaction of amino acids that may not necessarily be contained in a single stretch. Peptides of varying lengths (e.g., at least 4-6 amino acids long) can be isolated or synthesized (e.g., recombinantly) and used for binding assays with an IL-6 antagonist antibody. In another example, the epitope to which the IL-6 antagonist antibody binds can be determined in a systematic screening by using overlapping peptides derived from the IL-6 sequence and determining binding by the IL-6 antagonist antibody. According to the gene fragment expression assays, the open reading frame encoding IL-6 is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of IL-6 with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein in vitro, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled IL-6 fragments is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries) or yeast (yeast display). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. In an additional example, mutagenesis of an antigen, domain swapping experiments and alanine scanning mutagenesis can be performed to identify residues required, sufficient, and/or necessary for epitope binding. For example, alanine scanning mutagenesis experiments can be performed using a mutant IL-6 in which various residues of the IL-6 polypeptide have been replaced with alanine. By assessing binding of the antibody to the mutant IL-6, the importance of the particular IL-6 residues to antibody binding can be assessed.

[0247] In some embodiments, an isolated antagonist anti-IL-6 antibody is provided, wherein the antibody binds human IL-6 with an affinity of between about 0.01 pM to about 10 nM. In some embodiments, an isolated antagonist anti-IL-6 antibody is provided, wherein the antibody binds human IL-6 with an affinity of between about 0.1 pM to about 2 nM. In some embodiments, an isolated antagonist anti-IL-6 antibody is provided, wherein the antibody binds human IL-6 with an affinity of about 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 pM.

[0248] The binding affinity (K_D) of an IL-6 antagonist antibody to IL-6 can be about 0.001 to about 200 nM. In some embodiments, the binding affinity is any of about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, about 60 pM, about 50 pM, about 20 pM, about 15 pM, about 10 pM, about 5 pM, about 2 pM, or about 1 pM. In some embodiments, the binding affinity is less than any of about 250 nM, about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, about 50 pM, about 20 pM, about 10 pM, about 5 pM, about 2 pM, about 1 pM, about 0.5 pM, about 0.1 pM, about 0.05 pM, about 0.01 pM, about 0.005 pM, or about 0.001 pM.

[0249] In some embodiments, an isolated antagonist anti-IL-6 antibody is provided, wherein the antibody binds human IL-6 with a koff that is at least 5.0E-03 at 37 degrees. In some embodiments the koff is 5E-04. In some embodiments, an isolated antagonist anti-IL-6 antibody is provided, wherein the antibody binds human IL-6 with a koff that is better than 5.0E-04 at 37 degrees.

[0250] In some embodiments, binding affinity can be defined in terms of one or more of association constant (k_a), dissociation constant (k_d), and analyte concentration that achieves half-maximum binding capacity (KD). In some embodiments, k_a can range from about 0.50E+05 to about 5.00E+08. In some embodiments, k_d can range from about 0.50E-06 to about 5.00E-03. In some embodiments, K_D can range from about 0.50E-12 to about 0.50E-07.

[0251] In some embodiments, a pharmaceutical composition comprising any of the antibodies disclosed herein is provided. In some embodiments, a pharmaceutical composition comprising any of the conjugates disclosed herein is provided. In some embodiments, the pharmaceutical composition comprises one or more pharmaceutically acceptable carriers. In some embodiments, the pharmaceutical composition is a liquid. In some embodiments, the pharmaceutical composition has an endotoxin level less than about 0.2 EU/ml. In some embodiments, the pharmaceutical composition is a liquid and has an endotoxin level less than about 0.2 EU/ml. In some embodiments, the pharmaceutical composition is a liquid and has an endotoxin level less than about 2.0, 1, 0.5, 0.2 EU/ml. In some embodiments, for example in intravitreal injection, the endotoxin limit is 0.01-0.02 EU/injection/eye.

[0252] Some embodiments provide any of the following, or compositions (including pharmaceutical compositions) comprising an antibody having a partial light chain sequence and a

partial heavy chain sequence as found in Tables 1A and 1B, or variants thereof. In Tables 1A and 1B, the underlined sequences are some embodiments of CDR sequences as provided herein.

TABLE 1A.

Table 1A Anti-IL-6 heavy chain variable region sequences. CDRs are underlined.

ID	Sequence
I	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAISWVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRFTFS LDTSKSTAYLQMNSLRAEDTAVYYCARQLWGYYALDVWGQGTLVTVSS (SEQ ID NO: 89)
II	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAWSWVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCARQLWGYYALDVWGQGTLVTVSS (SEQ ID NO: 90)
IIa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMHWVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCARQAWGYYALDIWGQGTLVTVSS (SEQ ID NO: 256)
IIb	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMHWVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCARQSWGYYALDIWGQGTLVTVSS (SEQ ID NO: 257)
IIc	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMHWVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCARQGWGYYALDIWGQGTLVTVSS (SEQ ID NO: 258)
IId	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMHWVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCARQTWGYYALDIWGQGTLVTVSS (SEQ ID NO: 259)
IIe	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMHWVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCARQVWGYYALDIWGQGTLVTVSS (SEQ ID NO: 260)
IIf	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMHWVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCARQQWGYYALDIWGQGTLVTVSS (SEQ ID NO: 261)
IIg	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMHWVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCARQKWGYYALDIWGQGTLVTVSS (SEQ ID NO: 262)

Table 1B. Anti-IL-6 light chain variable region sequences. CDRs are underlined.

ID	Sequence
III	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQQKPGKAPKLLIYDDSSLASGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK(SEQ ID NO: 91)
IV	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQQKPGKAPKLLIYDASSLASGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK (SEQ ID NO: 92)
V	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQQKPGKAPKLLIYDDSNLASGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK (SEQ ID NO: 93)

[0253] In some embodiments, the antibody does not have one or more (or any) of the following CDRs, Table 1E (which includes Table 1E1, Table 1E2, and/or Table 1E3).

TABLE 1E1

		Sequence
Heavy chain	CDR1	GFTFSPFAMS (SEQ ID NO: 94)
	CDR2	KISPGGSWTYYSDTVTG (SEQ ID NO: 95)
	CDR3	QLWGYYALDI (SEQ ID NO: 171)
Light chain	CDR1	SASISVSYMY (SEQ ID NO: 96)
	CDR2	DMSNLAS(SEQ ID NO: 97)
	CDR3	MQWSGYPYT (SEQ ID NO: 98)

TABLE 1E2

		Sequence
Heavy chain	CDR1	PFAMS (SEQ ID NO: 244)
	CDR2	KISPGGSWTYYSDTVTG (SEQ ID NO: 245)
	CDR3	QLWGYYALDI (SEQ ID NO: 246)
Light chain	CDR1	SASISVSYMY (SEQ ID NO: 247)
	CDR2	DMSNLAS (SEQ ID NO: 248)
	CDR3	MQWSGYPYT (SEQ ID NO: 249)

Table 1E3

		Sequence
Heavy chain	CDR1	GFTFSPFAMS (SEQ ID NO: 250)
	CDR2	WVAKISPGGSWTYYSDTVTG (SEQ ID NO: 251)
	CDR3	ARQLWGYYALDI (SEQ ID NO: 252)
Light chain	CDR1	SASISVSYMY (SEQ ID NO: 253)
	CDR2	LLIYDMSNLAS (SEQ ID NO: 254)
	CDR3	MQWSGYPYT (SEQ ID NO: 255)

[0254] In some embodiments, a composition as disclosed herein comprises an antibody having a partial or complete light chain sequence and a partial or complete heavy chain sequence from any of the options provided in Tables 1A, 1B, 0.1 and/or 0.2, or variants thereof. In some embodiments, the antibody (or binding fragment thereof) can include any one or more of the CDRs provided in Tables 1A, 1B, 0.1 and/or 0.2. In some embodiments, the antibody (or binding fragment thereof) can include any three or more of the CDRs provided in Tables 1A, 1B, 0.1 and/or 0.2. In some embodiments, the antibody (or binding fragment thereof) can include any all six of the CDRs provided in Tables 1A, 1B, 0.1 and/or 0.2. In some embodiments, the heavy and/or light chain can be any one or more of the other antibody constructs provided herein, including, for example, those provided in FIGs. 5, 18, and/or 21-24 and Tables 0.1, 0.2, 1A, and/or 1E.

[0255] In some embodiments, a composition as disclosed herein comprises an antibody having a partial or complete light chain CDR sequence and a partial or complete heavy chain CDR sequence from any of the options provided in Tables 1A, 1B, 0.1 and/or 0.2.

[0256] In some embodiments, CDR portions of IL-6 antagonist antibodies are also provided. Determination of CDR regions is well within the skill of the art. It is understood that in some embodiments, CDRs can be a combination of the IMGT and Paratome CDRs (also termed "combined CDRs" or "extended CDRs"). Determination of CDRs is well within the skill of the art. In some embodiments, the CDRs are the IMGT CDRs. In other embodiments, the CDRs are the Paratome CDRs. In other embodiments, the CDRs are the extended, AbM, conformational, Kabat, or Chothia CDRs. In embodiments with more than one CDR, the CDRs may be any of IMGT, Paratome, extended, Kabat, Chothia, AbM, conformational CDRs, or combinations thereof. In some embodiments, other CDR definitions may also be used. In some embodiments, only residues that are in common between 2, 3, 4, 5, 6, or 7 of the above definitions are used (resulting in a shorter sequence). In some embodiments, any residue in any of 2, 3, 4, 5, 6, or 7 of the above definitions can be used (resulting in a longer sequence).

[0257] In some embodiments, an IL-6 antagonist antibody comprises three CDRs of any one of the heavy chain variable regions shown in Tables 1A, 1B, 0.1 and/or 0.2. In some embodiments, the antibody comprises three CDRs of any one of the light chain variable regions shown in Tables 1A, 1B, 0.1 and/or 0.2. In some embodiments, the antibody comprises three CDRs of any one of the heavy chain variable regions shown in Tables 1A, and three CDRs of any one of the light chain variable regions shown in Tables 1B. In some embodiments, the CDRs are one or more of those designated in Tables 0.1 (which includes Table 0.1A and/or 0.1B) or 0.2 (which includes Table 0.1A and/or 0.1B) below:

Table 0.1A ANTI IL-6 HEAVY CHAIN CDR SEQUENCES.

ID	CDR1	CDR2	CDR3
I	GFTFSPFAIS (SEQ ID NO: 99)	VAKISPGGSWTYYSDTVTD (SEQ ID NO: 100)	ARQLWGYYALDV (SEQ ID NO: 101)
II	GFTFSPFAWS (SEQ ID NO: 102)	VAKISPGGSWTYYSDTVTD (SEQ ID NO: 103)	ARQLWGYYALDV (SEQ ID NO: 104)
IIa	GFTFSPFAMH (SEQ ID NO: 49)	VAKISPGGSWTYYSDTVTD (SEQ ID NO: 50)	ARQAWGYYALDI (SEQ ID NO: 51)
IIb	GFTFSPFAMH (SEQ ID NO:52)	VAKISPGGSWTYYSDTVTD (SEQ ID NO:53)	ARQSWGYYALDI (SEQ ID NO:54)
IIc	GFTFSPFAMH (SEQ ID NO:55)	VAKISPGGSWTYYSDTVTD (SEQ ID NO: 56)	ARQGWGYYALDI (SEQ ID NO: 57)

IId	GFTFSPFAMH (SEQ ID NO: 58)	VAKISPGGSWTYYSDTVTD (SEQ ID NO: 59)	ARQTWGYYALDI (SEQ ID NO: 60)
IIe	GFTFSPFAMH (SEQ ID NO: 61)	VAKISPGGSWTYYSDTVTD (SEQ ID NO: 62)	ARQVWGYYALDI (SEQ ID NO: 63)
IIf	GFTFSPFAMH (SEQ ID NO: 64)	VAKISPGGSWTYYSDTVTD (SEQ ID NO: 65)	ARQQWGYYALDI (SEQ ID NO: 66)
IIg	GFTFSPFAMH (SEQ ID NO: 67)	VAKISPGGSWTYYSDTVTD (SEQ ID NO: 68)	ARQKWGYYALDI (SEQ ID NO: 69)

Table 0.1B ANTI IL-6 HEAVY CHAIN CDR SEQUENCES. Kabat

	CDR1	**CDR2**	**CDR3**
	PFAMH SEQ ID NO. 172)	KISPGGSWTYYSDTVTD SEQ ID NO. 173)	QAWGYYALDI SEQ ID NO. 174)
	PFAMH SEQ ID NO. 175)	KISPGGSWTYYSDTVTD SEQ ID NO. 176)	QSWGYYALDI SEQ ID NO. 177)
	PFAMH SEQ ID NO. 178)	KISPGGSWTYYSDTVTD SEQ ID NO. 179)	QGWGYYALDI SEQ ID NO. 180)
	PFAMH SEQ ID NO. 181)	KISPGGSWTYYSDTVTD SEQ ID NO. 182)	QTWGYYALDI SEQ ID NO. 183)
	PFAMH SEQ ID NO. 184)	KISPGGSWTYYSDTVTD SEQ ID NO. 185)	QVWGYYALDI SEQ ID NO. 186)
	PFAMH SEQ ID NO. 187)	KISPGGSWTYYSDTVTD SEQ ID NO. 188)	QQWGYYALDI SEQ ID NO. 189)
	PFAMH SEQ ID NO. 190)	KISPGGSWTYYSDTVTD SEQ ID NO. 191)	QKWGYYALDI SEQ ID NO. 192)
	PFAIS SEQ ID NO. 193)	KISPGGSWTYYSDTVTD SEQ ID NO. 194)	QLWGYYALDV SEQ ID NO. 195)
	PFAWS SEQ ID NO. 196)	KISPGGSWTYYSDTVTD SEQ ID NO. 197)	QLWGYYALDV SEQ ID NO. 198)

Table 0.2A. ANTI IL-6 LIGHT CHAIN CDR SEQUENCES.

ID	**CDR1**	**CDR2**	**CDR3**
III	SASISVSYLY (SEQ ID NO: 105)	LLIYDDSSLAS (SEQ ID NO: 106)	QQWSGYPYT (SEQ ID NO: 107)
IV	SASISVSYLY (SEQ ID NO: 108)	LLIYDASSLAS (SEQ ID NO: 109)	QQWSGYPYT (SEQ ID NO: 110)
V	SASISVSYLY (SEQ ID NO: 111)	LLIYDDSNLAS (SEQ ID NO: 112)	QQWSGYPYT (SEQ ID NO: 113)

Table 0.2B ANTI IL-6 LIGHT CHAIN CDR SEQUENCES.

	CDR1	**CDR2**	**CDR3**
	SASISVSYLY SEQ ID NO. 199)	DDSSLAS SEQ ID NO. 200)	QQWSGYPYT SEQ ID NO. 201)
	SASISVSYLY SEQ ID NO. 202)	DASSLAS SEQ ID NO. 203)	QQWSGYPYT SEQ ID NO. 204)
	SASISVSYLY SEQ ID NO. 205)	DDSNLAS SEQ ID NO. 206)	QQWSGYPYT SEQ ID NO. 207)

[0258] In some embodiments, the antibody used for binding to IL-6 can be one that includes one or more of the sequences in Tables 1A, 1B, 0.1 and/or 0.2. In some embodiments, the antibody used for binding to IL-6 can be one that includes three or more of the sequences in Tables 1A, 1B, 0.1 and/or 0.2. In some embodiments, the antibody used for binding to IL-6 can be one that includes six of the sequences in any one of Tables 1A, 1B, 0.1 and/or 0.2. In some embodiments, the antibody that binds to IL-6 can be one that competes for binding with an antibody that includes 6 of the specified CDRs in any one of Tables 1A, 1B, 0.1 and/or 0.2.

[0259] In some embodiments, the antibody can be linked or fused to a VEGF Trap sequence. In some embodiments, this Trap sequence can be as shown in Table 1C. In some embodiments, the sequence is at least 80% identical to that shown in Table 1C, e.g, at least 80, 85, 90, 95, 96, 97, 98, 99% identical to that shown in 1C. In some embodiments, any of the VEGF Trap molecules in U.S. Pub. No. 20150376271 can be employed herein. In some embodiments, the VEGF Trap sequence is fused to IL-6 in one of the following manners: to an N-terminal end of a heavy chain comprising IL-6 VH (FIG. 6 left), or between a hinge region and after a CH1 domain of a heavy chain comprising IL-6 VH (FIG. 6 right). Unless designated otherwise, both options in the alternative and together are contemplated for the embodiments provided herein wherein any Ab-Trap fusion is discussed. In some embodiments, the term "Trap" refers to a full length extracellular region or any portion thereof, or combination of portions from different VEGF receptors that can antagonize signaling between at least one VEGF and VEGFR. Preferably, the extracellular trap segment includes at least one domain from one of VEGFR-1, -2 or -3, and more preferably at least two contiguous domains, such as D2 and D3. Optionally, an extracellular domain includes at least one domain from at least two different VEGFRs. A preferred extracellular domain comprises or consists essentially of D2 of VEGFR-1 and D3 of VEGFR-2.

TABLE 1C. VEGFR1, DOMAIN 2 AND VEGFR2, DOMAIN 3 FUSION SEQUENCE

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVN
GHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEM
KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK (SEQ ID NO: 114)

[0260] In some embodiments, the IL-6 Ab VEGF Trap construct can have any of the sequences provided in TABLE 1D. In some embodiments, the construct can be at least identical

to the sequences in Table 1D, e.g., 80, 85, 90, 95, 96, 97, 98, 99 or higher. In some embodiments, the fusion protein can be in line with those percentages, with the exception that the antibody IL-6 domain does not contain one or more of the CDRs in Table 1E. In some embodiments, the fusion protein is one that contains one or more of the identified sequences in FIG. 5, e.g., one of more of the CDRS (including 2, 3, 4, 5 or 6 of the boxed CDRs) and/or the entire heavy and light chain variable regions, along with a VEGF Trap sequence (e.g., Table 1C). In some embodiments, the sequences can be directly fused to one another. In some embodiments, one or more flexible linking sequences or sections can be used. The linking sequence can be positioned between the Ab sequence and the VEGF Trap sequence. These sequences can be 5 to 30 amino acids in length. In some embodiments, the linking sequence can include G and S in a ratio of about 4:1. In some embodiments, the linker includes the following sequence: GGGGSGGGGS (SEQ ID NO: 115). In some embodiments, any flexible linker can be employed. In some embodiments, the Fc portion of the Il-6 Ab is IgG1.

TABLE 1D.

HEAVY AND LIGHT CHAIN SEQUENCES FOR DUAL INHIBITOR MOLECULES. CDRS ARE UNDERLINED IN THE HEAVY AND LIGHT CHAINS, VEGF TRAP SEQUENCE IS BOLDED IN BLACK, GLY-SER LINKER IS ITALICIZED.

ID	Heavy chain	Light chain
A	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK***GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAISWVRQAPGKGLEWVAKISPGGS WTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAED TAVYYCARQLWGYYALDVWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 116)**	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 117)

B	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK***GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAISWVRQAPGKGLEWVAKISPGGS WTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAED TAVYYCARQLWGYYALDVWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 118)**	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDASSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 119)
C	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK***GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAISWVRQAPGKGLEWVAKISPGGS WTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAED TAVYYCARQLWGYYALDVWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 120)**	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 121)
D	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK***GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAWSWVRQAPGKGLEWVAKISPGG SWTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAE DTAVYYCARQLWGYYALDVWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSCSPGK (SEQ ID NO: 122)**	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 123)
E	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT**	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDASSLASGVPSRFSGSGSGTDFTL

	YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK*GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAWSWVRQAPGKGLEWVAKISPGG SWTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAE DTAVYYCARQLWGYYALDVWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSCSPGK (SEQ ID NO: 124)**	TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 125)
F	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK***GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAWSWVRQAPGKGLEWVAKISPGG SWTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAE DTAVYYCARQLWGYYALDVWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSCSPGK (SEQ ID NO: 126)**	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 127)
G	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK***GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAMHWVRQAPGKGLEWVAKISPGG SWTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAE DTAVYYCARQAWGYYALDIWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSCSPGK (SEQ ID NO: 263)**	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 117)
H	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN**	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDASSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA

	WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK*GGGGSGGGGS*EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAMHWVRQAPGKGLEWVAKISPGG SWTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAE DTAVYYCARQAWGYYALDIWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSCSPGK (SEQ ID NO: 263)	KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 119)
I	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK***GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAMHWVRQAPGKGLEWVAKISPGG SWTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAE DTAVYYCARQAWGYYALDIWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSCSPGK (SEQ ID NO: 263)**	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 121)
J	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAIS WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQLWG YYALDVWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC*GGGGSGGGGS***SDTGRPFVE MYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKF PLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTC EATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIE LSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQ HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSD QGLYTCAASSGLMTKKNSTFVRVHEK**DKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 128)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 129)
K	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAIS WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQLWG YYALDVWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDASSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST

	PSNTKVDKKVEPKSC*GGGGSGGGGS***SDTGRPFVE** **MYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKF** **PLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTC** **EATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIE** **LSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQ** **HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSD** **QGLYTCAASSGLMTKKNSTFVRVHEK**DKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 130)	LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 131)
L	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAIS WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQLWG YYALDVWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC*GGGGSGGGGS***SDTGRPFVE** **MYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKF** **PLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTC** **EATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIE** **LSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQ** **HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSD** **QGLYTCAASSGLMTKKNSTFVRVHEK**DKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 132)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 133)
M	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAWS WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQLWG YYALDVWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC*GGGGSGGGGS***SDTGRPFVE** **MYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKF** **PLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTC** **EATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIE** **LSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQ** **HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSD** **QGLYTCAASSGLMTKKNSTFVRVHEK**DKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 134)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 135)
N	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAWS WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQLWG YYALDVWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC*GGGGSGGGGS***SDTGRPFVE** **MYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKF**	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDASSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 137)

	PLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTC EATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIE LSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQ HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSD QGLYTCAASSGLMTKKNSTFVRVHEKDKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 136)	
O	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAWS WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQLWG YYALDVWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC*GGGGSGGGGS***SDTGRPFVE MYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKF PLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTC EATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIE LSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQ HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSD QGLYTCAASSGLMTKKNSTFVRVHEK**DKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 138)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 139)
P	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMH WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQAW GYYALDIWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSC*GGGGSGGGGS***SDTGRPFV EMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKK FPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLT CEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGI ELSVGEKLVLNCTARTELNVGIDFNWEYPSSKH QHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRS DQGLYTCAASSGLMTKKNSTFVRVHEK**DKTHT CPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSCSPGK (SEQ ID NO: 266)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 117)
Q	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMH WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQAW GYYALDIWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSC*GGGGSGGGGS***SDTGRPFV EMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKK FPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLT CEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGI	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDASSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 119)

	ELSVGEKLVLNCTARTELNVGIDFNWEYPSSKH QHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRS DQGLYTCAASSGLMTKKNSTFVRVHEKDKTHT CPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSCSPGK (SEQ ID NO: 267)	
R	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMH WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQAW GYYALDIWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSC*GGGGSGGGGS***SDTGRPFV EMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKK FPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLT CEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGI ELSVGEKLVLNCTARTELNVGIDFNWEYPSSKH QHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRS DQGLYTCAASSGLMTKKNSTFVRVHEK**DKTHT CPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSCSPGK (SEQ ID NO: 268)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 121)

[0261] To express the anti-IL-6 antibodies and/or IL-6 VEGF Traps provided herein, DNA fragments encoding VH and VL regions described can first be obtained. Various modifications, e.g. mutations, deletions, and/or additions can also be introduced into the DNA sequences using standard methods known to those of skill in the art. For example, mutagenesis can be carried out using standard methods, such as PCR-mediated mutagenesis, in which the mutated nucleotides are incorporated into the PCR primers such that the PCR product contains the desired mutations or site-directed mutagenesis.

[0262] The invention encompasses modifications to the variable regions shown herein. For example, the invention includes antibodies comprising functionally equivalent variable regions and CDRs which do not significantly affect their properties as well as variants which have enhanced or decreased activity and/or affinity. For example, the amino acid sequence may be mutated to obtain an antibody with the desired binding affinity to IL-6. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously

change the functional activity, or which mature (enhance) the affinity of the polypeptide for its ligand, or use of chemical analogs.

[0263] 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 intra-sequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the half-life of the antibody in the blood circulation.

[0264] Substitution variants have at least one amino acid residue in the antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but framework alterations are also contemplated. Conservative substitutions are shown in Table 0.4 under the heading of "conservative substitutions." If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 0.4, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 0.4 - Amino Acid Substitutions

Original Residue	Conservative Substitutions	Exemplary Substitutions
Ala (A)	Val	Val; Leu; Ile
Arg (R)	Lys	Lys; Gln; Asn
Asn (N)	Gln	Gln; His; Asp, Lys; Arg
Asp (D)	Glu	Glu; Asn
Cys (C)	Ser	Ser; Ala
Gln (Q)	Asn	Asn; Glu
Glu (E)	Asp	Asp; Gln
Gly (G)	Ala	Ala
His (H)	Arg	Asn; Gln; Lys; Arg
Ile (I)	Leu	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Ile	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn

Original Residue	Conservative Substitutions	Exemplary Substitutions
Met (M)	Leu	Leu; Phe; Ile
Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr; Phe
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; Norleucine

[0265] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a β-sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

[0266] (1) Non-polar: Norleucine, Met, Ala, Val, Leu, Ile;

[0267] (2) Polar without charge: Cys, Ser, Thr, Asn, Gln;

[0268] (3) Acidic (negatively charged): Asp, Glu;

[0269] (4) Basic (positively charged): Lys, Arg;

[0270] (5) Residues that influence chain orientation: Gly, Pro; and

[0271] (6) Aromatic: Trp, Tyr, Phe, His.

[0272] Non-conservative substitutions are made by exchanging a member of one of these classes for another class.

[0273] One type of substitution, for example, that may be made is to change one or more cysteines in the antibody, which may be chemically reactive, to another residue, such as, without limitation, alanine or serine. For example, there can be a substitution of a non-canonical cysteine. The substitution can be made in a CDR or framework region of a variable domain or in the constant region of an antibody. In some embodiments, the cysteine is canonical. Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent

aberrant cross-linking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability, particularly where the antibody is an antibody fragment such as an Fv fragment.

[0274] The antibodies may also be modified, e.g. in the variable domains of the heavy and/or light chains, e.g., to alter a binding property of the antibody. Changes in the variable region can alter binding affinity and/or specificity. In some embodiments, no more than one to five conservative amino acid substitutions are made within a CDR domain. In other embodiments, no more than one to three conservative amino acid substitutions are made within a CDR domain. For example, a mutation may be made in one or more of the CDR regions to increase or decrease the K_D of the antibody for IL-6, to increase or decrease k_{off}, or to alter the binding specificity of the antibody. Techniques in site-directed mutagenesis are well-known in the art. See, e.g., Sambrook et al. and Ausubel et al., supra.

[0275] According to an aspect, the IgG domain of an IL-6 antagonist antibody can be IgG1, IgG2, IgG3 or IgG4. According to another aspect, the IgG domain can be a composite in which a constant regions is formed from more than one of the above isotypes (e.g., CH_1 region from IgG2 or IgG4, hinge, CH_2 and CH_3 regions from IgG1). In choosing an isotype, it is known in the art that human isotopes IgGl and IgG3 have complement-mediated cytotoxicity whereas human isotypes IgG2 and IgG4 have poor or no complement-mediated cytotoxicity. In some embodiments the IL-6 antagonist antibody isotype is IgG1.

[0276] The light chain constant region can be either human lambda or kappa. In some embodiments, the IL-6 antagonist antibody has a human kappa light chain constant region.

[0277] Human constant regions show allotypic variation and isoallotypic variation between different individuals, that is, the constant regions can differ in different individuals at one or more polymorphic positions. Isoallotypes differ from allotypes in that sera recognizing an isoallotype binds to a non-polymorphic region of one or more other isotypes. Reference to a human constant region includes a constant region with any natural allotype or any permutation of residues occupying polymorphic positions in natural allotypes or up to 3, 5 or 10 substitutions for reducing or increasing effector function as described below.

[0278] One or several amino acids at the amino or carboxy terminus of the light and/or heavy chains such as the C-terminal lysine of the heavy chain, may be missing or derivatized in a proportion or all of the molecules.

[0279] Substitutions can be made in the constant regions to reduce or increase effector function such as complement-mediated cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) (see, e.g., Winter et al., US Patent No.5,624,821; Tso et al., US Patent No. 5,834,597; and Lazar et al., Proc. Natl. Acad. Sci. USA 103:4005, 2006), or to prolong half-life in humans (see, e.g., Hinton et al., J. Biol. Chem. 279:6213, 2004).

[0280] In some embodiments, the IL-6 antagonist antibodies provided herein include one more substitutions that reduce complement mediated cytotoxicity. Reduction in complement mediated cytotoxicity can be accomplished with or without reduction in Fc receptor binding depending on the nature of the mutation(s). Antibodies with reduced complement mediated cytotoxicity but little or no reduction in Fc receptor allow a desired effect of Fc-mediated phagocytosis of iC3b without activating complement, which may contribute to side effects. Exemplary mutations known to reduce complement-mediated cytotoxicity in human constant regions include mutations at positions 241, 264, 265, 270, 296, 297, 322, 329 and 331 by EU numbering. Mutations in positions 318, 320, and 322 have been reported to reduce complement activation in mouse antibodies. Alanine is a preferred residue to occupy these positions in a mutated constant region. Some exemplary human mutations that have been used include F241A, V264A, D265A, V296A, N297A, K322A, and P331S in human IgG3 and D270A or E, N297Q, K322A, P329A, and P331S in human IgGl (EU numbering).

[0281] Here, as elsewhere, the EU numbering scheme is used for numbering amino acids in the constant region of an antibody. When a residue in a variable region is referenced herein (unless designated otherwise) the residue numbering is according to the variable domain (or, if designated, the SEQ ID NO). Substitution at any or all of positions 234, 235, 236 and/or 237 reduce affinity for Fcγ receptors, particularly FcγRI receptor and also reduces complement binding and activation *(see, e.g.,* US 6,624,821 WO/2009/052439). An alanine substitution at positions 234, 235 and 237 reduces effector functions, particularly in the context of human IgGl. Optionally, positions 234, 236 and/or 237 in human IgG2 are substituted with alanine and position 235 with glutamine. *(See, e.g.,* US 5,624,821) to reduce Fc receptor binding. Exemplary substitutions for increasing half-life include a Gln at position 250 and/or a Leu at position 428. In accordance with an aspect of the present invention, where the anti-IL-6 antibody presented has a human IgG1 isotype, it is preferred that the antibody has at least one mutation in the constant region. Preferably, the mutation reduces complement fixation or activation by the constant region. In particularly

preferred aspects of the present invention, the antibody has one or more mutations at positions E233, L234, L235, G236, G237, A327, A330 and P331 by EU numbering. Still more preferably, the mutations constitute one or more of the following E233P, L234V, L234A, L235A, G237A, A327G, A330S and P331S by EU numbering. In the most preferred embodiments the human IgG1 has the following mutations L234A, L235A and G237A by EU numbering.

Conjugates

[0282] The half-life of IL-6 antagonist antibodies and/or IL-6 Ab VEGF Traps can be extended by attachment of a "half-life extending moieties" or "half-life extending groups," which terms are herein used interchangeably to refer to one or more chemical groups attached to one or more amino acid site chain functionalities such as -SH, -OH, -COOH, -CONH2, -NH2, or one or more N- and/or O-glycan structures and that can increase *in vivo* circulatory half-life of proteins/peptides when conjugated to these proteins/peptides. Examples of half-life extending moieties include polymers described herein, particularly those of zwitterionic monomers, such as HEMA-phosphorylcholine, PEG, biocompatible fatty acids and derivatives thereof, Hydroxy Alkyl Starch (HAS) e.g. Hydroxy Ethyl Starch (HES), Poly Ethylene Glycol (PEG), Poly (Gly_x-Ser_y) (HAP), Hyaluronic acid (HA), Heparosan polymers (HEP), Fleximers, Dextran, Poly-sialic acids (PSA), Fc domains, Transferrin, 25 Albumin, Elastin like (ELP) peptides, XTEN polymers, PAS polymers, PA polymers, Albumin binding peptides, CTP peptides, FcRn binding peptides and any combination thereof.

[0283] In some embodiments, the antibody is conjugated with a phosphorylcholine containing polymer. In some embodiments, the antibody is conjugated with a poly(acryloyloxyethyl phosphorylcholine) containing polymer, such as a polymer of acrylic acid containing at least one acryloyloxyethyl phosphorylcholine monomer such as 2-methacryloyloxyethyl phosphorylcholine (i.e., 2-methacryloyl-2'-trimethylammonium ethyl phosphate).

[0284] In some embodiments, the antibody and/or antibody VEGF Trap fusion is conjugated with a water-soluble polymer, which refers to a polymer that is soluble in water. A solution of a water-soluble polymer may transmit at least about 75%, more preferably at least about 95% of light, transmitted by the same solution after filtering. On a weight basis, a water-soluble

polymer or segment thereof may be at least about 35%, at least about 50%, about 70%, about 85%, about 95% or 100% (by weight of dry polymer) soluble in water.

[0285] In one embodiment a half-life extending moiety can be conjugated to an IL-6 antagonist antibodies and/or IL-6 Ab VEGF Trap via free amino groups of the protein using N-hydroxysuccinimide (NHS) esters. Reagents targeting conjugation to amine groups can randomly react to ϵ-amine group of lysines, α-amine group of N-terminal amino acids, and δ-amine group of histidines.

[0286] However, IL-6 antagonist antibodies of the present invention have many amine groups available for polymer conjugation. Conjugation of polymers to free amino groups, thus, might negatively impact the ability of the antibody to bind to the epitope.

[0287] In another embodiment, a half-life extending moiety is coupled to one or more free SH groups using any appropriate thiol-reactive chemistry including, without limitation, maleimide chemistry, or the coupling of polymer hydrazides or polymer amines to carbohydrate moieties of the IL-6 antagonist antibodies and/or IL-6 Ab VEGF Traps after prior oxidation. The use of maleimide coupling is a particularly preferred embodiment of the present invention. Coupling preferably occurs at cysteines naturally present or introduced via genetic engineering.

[0288] In some embodiments, polymers are covalently attached to cysteine residues introduced into IL-6 antagonist antibodies and/or IL-6 Ab VEGF Traps by site directed mutagenesis. In some embodiments, the cysteine residues in the Fc portion of the IL-6 antagonist antibody and/or IL-6 Ab VEGF Trap can be used. In some embodiments, sites to introduce cysteine residues into an Fc region are provided in WO 2013/093809, US 7,521,541, WO 2008/020827, US 8,008,453, US 8,455,622 and US2012/0213705, incorporated herein by reference for all purposes. In some embodiments, cysteine mutations are Q347C and L443C referring to the human IgG heavy chain by the EU index of Kabat. In some embodiments, the cysteine added by directed mutagenesis for subsequent polymer attachment is L443C. In some embodiments, the stoichiometry of IL-6 antagonist antibody to polymer is 1:1; in other words, a conjugate consists essentially of molecules each comprising one molecule of IL-6 antagonist antibody and/or IL-6 Ab VEGF Trap conjugated to one molecule of polymer. In some embodiments, coupling can occur at one or more lysines.

[0289] In some embodiments, a conjugate comprises an isolated antagonist antibody that specifically binds to IL-6 or IL-6 Ab VEGF Trap is conjugated to a polymer. In some

embodiments, the polymer comprises a zwitterionic monomer. In some embodiments, the zwitterionic monomer, without limitations, is HEMA-phosphorylcholine, PEG, biocompatible fatty acids and derivatives thereof, Hydroxy Alkyl Starch (HAS) e.g. Hydroxy Ethyl Starch (HES), Poly Ethylene Glycol (PEG), Poly (Glyx-Sery) (HAP), Hyaluronic acid (HA), Heparosan polymers (HEP), Fleximers, Dextran, Poly-sialic acids (PSA), Fc domains, Transferrin, 25 Albumin, Elastin like (ELP) peptides, XTEN polymers, PAS polymers, PA polymers, Albumin binding peptides, CTP peptides, or FcRn binding peptides. In some embodiments, the polymer comprising a zwitterionic monomer is a half-life extending moiety.

[0290] In some embodiments, the IL-6 antagonist antibody and/or IL-6 Ab VEGF Trap can have a half-life extending moiety attached. In some embodiments, the half-life extending moiety is a zwitterionic polymer but PEG or other half-life extenders discussed below can alternatively be used. In some embodiments, the zwitterionic polymer is formed of monomers having a phosphorylcholine group. In some embodiments, the monomer is 2-(acryloyloxyethyl)-2'-(trimethylammoniumethyl) phosphate. In some embodiments, the monomer is 2-(methacryloyloxyethyl)-2'-(trimethylammoniumethyl) phosphate (HEMA-PC).

[0291] In some embodiments, the polymer conjugated to the IL-6 antagonist antibody and/or IL-6 Ab VEGF Trap has at least 2 or 3 or more arms. Some polymers have 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms. In some embodiments, the polymer has 3, 6 or 9 arms. In some embodiments, the polymer has 9 arms. In some embodiments, the polymer peak molecular weight is between 300,000 and 1,750,000 Da. In some embodiments, the polymer has a peak molecular weight between 500,000 and 1,000,000 Da. In some embodiments, the polymer has a peak molecular weight between 600,000 to 800,000 Da.

[0292] In some embodiments, a conjugate of antagonistic anti-IL-6 antibody and/or IL-6 Ab VEGF Trap and a polymer is provided. In some embodiments, the polymer has a peak molecular weight between 300,000 and 1,750,000 Daltons as measured by size exclusion chromatography – multi angle light scattering (hereinafter "SEC-MALS"). In some embodiments, the polymer has a peak molecular weight between 500,000 and 1,000,000 Daltons as measured by SEC-MALS. In some embodiments, the polymer has a peak molecular weight between 600,000 to 800,000 Daltons as measured by SEC-MALS.

[0293] In some embodiments, a half-life extending moiety may be conjugated to a naturally occurring cysteine residue of an IL-6 antagonist antibody and/or IL-6 Ab VEGF Trap

provided herein. In some embodiments, the half-life extending moiety is conjugated added to a cysteine that is added via site directed mutagenesis. In some embodiments, the cysteine is added by using recombinant DNA technology to add the peptide SGGGC or CAA to the C-terminus of either the light or heavy chain. In some embodiments, the peptide is added to the heavy chain. In some embodiments, the cysteine residue introduced via recombinant DNA technology is selected from the group consisting of (EU numbering) Q347C and L443C.

[0294] In accordance with another aspect of the present invention, a pharmaceutical composition is presented having an IL-6 antagonist antibody and/or IL-6 Ab VEGF Trap and a pharmaceutically acceptable excipient.

[0295] IL-6 antagonist antibodies and/or IL-6 Ab VEGF Traps (or other constructs provided herein) can be produced by recombinant expression including (i) the production of recombinant DNA by genetic engineering, (ii) introducing recombinant DNA into prokaryotic or eukaryotic cells by, for example and without limitation, transfection, electroporation or microinjection, (iii) cultivating the transformed cells, (iv) expressing anti-IL-6 antibodies, *e.g.* constitutively or on induction, and (v) isolating the anti-IL-6 antibody, *e.g.* from the culture medium or by harvesting the transformed cells, in order to (vi) obtain purified anti-IL-6 antibody.

[0296] In some embodiments, a conjugate comprising an anti-IL-6 antibody and/or IL-6 Ab VEGF Trap and a polymer is provided (as well as the other constructs provided herein, such as a bioconjugate). The antibody and/or Ab-Trap is any one of the antibodies and/or Traps disclosed herein. In some embodiments, the antibody is an IgG. In some embodiments, the antibody is IgA, IgE, IgD or IgM. In some embodiments, the polymer is covalently bonded to a sulfhydryl group. In some embodiments, the polymer is covalently bonded to a sulfhydryl group from a cysteine residue. In some embodiments, the polymer is covalently bonded to a sulfhydryl group from a cysteine residue on the heavy chain. In some embodiments, the polymer is covalently bonded to a sulfhydryl group from a cysteine residue on the heavy chain of the IgG. In some embodiments, the antibody comprises a cysteine residue at position 347 or 443 (EU numbering). In some embodiments, the polymer is covalently bonded to a sulfhydryl group from a cysteine residue at position 347 or 443 (EU numbering).

[0297] IL-6 antagonist antibodies and/or IL-6 Ab VEGF Trap can be produced by expression in a suitable prokaryotic or eukaryotic host system characterized by producing a pharmacologically acceptable anti-IL-6 antibody molecule. Examples of eukaryotic cells are

mammalian cells, such as CHO, COS, HEK 293, BHK, SK-Hip, and HepG2. Other suitable expression systems are prokaryotic (e.g., E. coli with pET/BL21 expression system), yeast (Saccharomyces cerevisiae and/or Pichia pastoris systems), and insect cells. In some embodiments, an isolated cell line that produces any of the antibodies and/or Ab-Traps disclosed herein is provided. In some embodiments, the isolated cell line is selected, without limitations, from one or more of CHO, k1SV, XCeed, CHOK1SV, GS-KO.

[0298] In some embodiments, an isolated nucleic acid encoding any of the antibodies and/or Ab-Traps disclosed herein is provided. In some embodiments, a recombinant expression vector comprising the isolated nucleic acid is provided. In some embodiments, a host cell comprises the expression vector.

[0299] A wide variety of vectors can be used for the preparation of the IL-6 antagonist antibodies and/or IL-6 Ab VEGF Traps and may be selected from eukaryotic and prokaryotic expression vectors. Examples of vectors for prokaryotic expression include plasmids such as, and without limitation, preset, pet, and pad, wherein the promoters used in prokaryotic expression vectors include one or more of, and without limitation, lac, trc, trp, recA, or araBAD. Examples of vectors for eukaryotic expression include, without limitation: (i) for expression in yeast, vectors such as, and without limitation, pAO, pPIC, pYES, or pMET, using promoters such as, and without limitation, AOX1, GAP, GAL1, or AUG1; (ii) for expression in insect cells, vectors such as and without limitation, pMT, pAc5, pIB, pMIB, or pBAC, using promoters such as and without limitation PH, p10, MT, Ac5, OpIE2, gp64, or polh, and (iii) for expression in mammalian cells, vectors such as, and without limitation, pSVL, pCMV, pRc/RSV, pcDNA3, or pBPV, and vectors derived from, in one aspect, viral systems such as and without limitation vaccinia virus, adeno-associated viruses, herpes viruses, or retroviruses, using promoters such as and without limitation CMV, SV40, EF-1, UbC, RSV, ADV, BPV, and beta-actin.

[0300] In some embodiments, a method of producing an IL-6 antagonist antibody and/or IL-6 Ab VEGF Trap is provided. In some embodiments, the method comprises culturing a cell line that recombinantly produces any of the antibodies and/or Ab-Traps disclosed herein under conditions wherein the antibody is produced and recovered. In some embodiments, a method of producing an IL-6 antagonist antibody and/or IL-6 Ab VEGF Trap is provided. In some embodiments, the method comprises culturing a cell line comprising nucleic acid encoding an antibody and/or Ab-Trap comprising a heavy chain comprising the amino acid sequence shown in

Table 1A and a light chain comprising the amino acid sequence shown in Table 1B under conditions wherein the antibody and/or IL-6 Ab VEGF Trap is produced and recovered. In some embodiments, the heavy and light chains of the antibody and/or IL-6 Ab VEGF Trap are encoded on separate vectors. In some embodiments, the heavy and light chains of the antibody and/or IL-6 Ab VEGF Trap are encoded on the same vector.

[0301] In accordance with another aspect of the present invention, provided are methods for synthesizing a zwitterionic polymer-IL-6 antagonist antibody and/or IL-6 Ab VEGF Trap conjugates, the conjugate having one or more functional agents and one or more polymer arms wherein each of the polymer arms has one or more monomer units wherein at least one of the units has a zwitterion. For example, such a method can have the steps of:

~~1.~~a. providing an initiator having one or more sites for monomer polymerization and a first linker having an amine group wherein the initiator is a trifluoro acetic acid salt;

~~2.~~b. providing one or more monomers suitable for polymerization wherein at least one of the monomers is zwitterionic;

~~3.~~c. reacting the monomers with the initiator to form one or more polymer arms each corresponding to the sites for monomer polymerization to provide an initiator-polymer conjugate having the first linker with the amine group;

~~4.~~d. providing a second linker having at least second and third reactive groups;

~~5.~~e. coupling one of the second and third reactive groups of the second linker to the amine group of the first linker of the initiator-polymer conjugate to provide a linker-initiator-polymer conjugate having one or more reactive groups that were not used in the coupling step; and

~~6.~~f. coupling one or more functional agents to one or more of the unreacted reactive groups of the linker-initiator-polymer moiety to provide the polymer-functional agent conjugate.

[0302] In some embodiments, a conjugate comprising an isolated antagonist antibody and/or IL-6 Ab VEGF Trap that specifically binds to IL-6 and a phosphorylcholine-containing polymer is provided. In some embodiments, the polymer is covalently bonded to the antibody and/or Ab-Trap. In some embodiments, the polymer is non-covalently bonded to the antibody and/or Ab-Trap.

[0303] In some embodiments, a conjugate comprising an anti-IL-6 antibody and/or IL-6 Ab VEGF Trap and a phosphorylcholine containing polymer is provided. In some embodiments, the polymer is covalently bonded to the antibody outside a variable region of the antibody. In some embodiments, a conjugate comprising an anti-IL-6 antibody and/or IL-6 Ab VEGF Trap and a phosphorylcholine containing polymer is provided. The polymer is covalently bonded to the antibody at a cysteine outside a variable region of the antibody. In some embodiments, a conjugate comprising an anti-IL-6 antibody and/or IL-6 Ab VEGF Trap and a phosphorylcholine containing polymer is provided, wherein the polymer is covalently bonded to the antibody at a cysteine outside a variable region of the antibody wherein said cysteine has been added via recombinant DNA technology. In some embodiments of the conjugate, the polymer comprises 2(methacryloyloxy)ethyl (2-(trimethylammonio)ethyl) phosphate (MPC) monomers.

[0304] In accordance with another aspect of the present invention, a method is provided where the conjugation group (e.g. maleimide) is added after polymer synthesis. This is sometimes referred to as a "snap-on strategy" or "universal polymer strategy". See, e.g., U.S. Patent Application No. 14/916,180 (published as U.S. Patent Application Publication No. 20160199501), hereby incorporated by reference in its entirety. In some embodiments, a single initiator moiety can be used for large scale polymer synthesis. Thus, conditions can be developed for scaled up optimal polymer synthesis. Such polymers can then be adapted to various types of functional agents by "snapping-on" various types of linkers. For example, if it is desired to conjugate a larger functional agent to a polymer of the instant invention such as an antibody of even a Fab fragment, a longer linker sequence can be snapped on to the polymer. In contrast, smaller functional agents may call for relatively shorter linker sequences.

[0305] In some embodiments of the methods, the initiator has about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 sites for polymer initiation. In some embodiments, the initiator has about 3, about 6, or about 9 sites for polymer initiation.

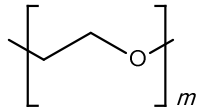
[0306] In accordance with another aspect of the present invention, a second linker has second, third, fourth, fifth, and sixth reactive groups. More preferably, a second linker has just second and third reactive groups.

[0307] In accordance with an aspect of the present invention, each polymer arm has from about 20 to about 2000 monomer units. Preferably, each arm has from about 100 to 500

monomer units or from about 500 to 1000 monomer units or from about 1000 to 1500 monomer units or from about 1500 to 2000 monomer units.

[0308] In accordance with an aspect of the present invention, the peak molecular weight of the polymer-functional agent conjugate is about 100,000 to 1,500,000 Da. Preferably, the peak molecular weight of the polymer-functional agent conjugate is about 200,000 to about 300,000 Da, about 400,000 to about 600,000 Da or about 650,000 to about 850,000 Da.

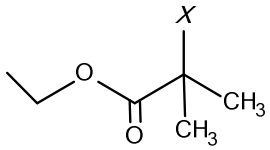
[0309] In accordance with another aspect of the present invention, the first linker is preferably alkyl, substituted alkyl, alkylene, alkoxy, carboxyalkyl, haloalkyl, cycloalkyl, cyclic alkyl ether, alkenyl, alkenylene, alkynyl, alkynylene, cycloalkylene, heterocycloalkyl, heterocycloalkylene, aryl, arylene, arylene-oxy, heteroaryl, amino, amido or any combination thereof. More preferably, the first linker has the formula:



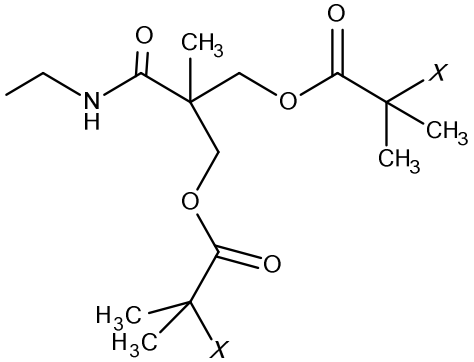
Formula (1)

wherein m is 1 to 10. In some embodiments, the first linker has the above formula (Formula (1)) and m is 4.

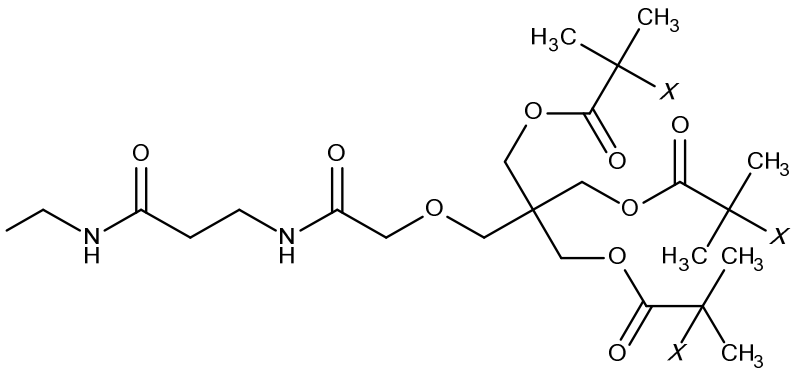
[0310] In some embodiments, the initiator preferably includes a structure selected from group consisting of



Formula (2)



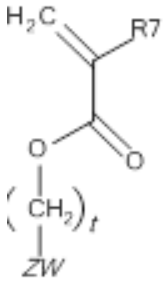
Formula (3), and



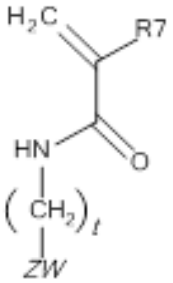
Formula (4)

wherein X is selected from the group consisting of NCS, F, Cl, Br and I. More preferably, X in Formula (2), Formula (3) and/or Formula (4) is Br.

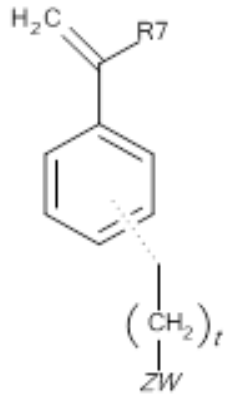
[0311] In some embodiments, the monomer is selected from the group consisting of



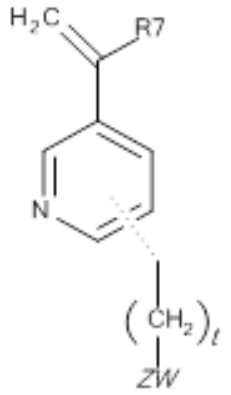
Formula (5)



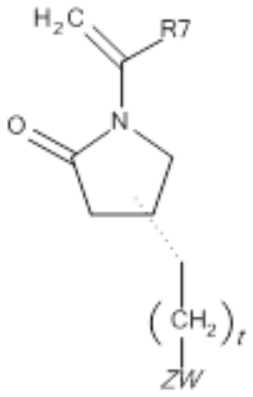
Formula (6)



Formula (7)



Formula (8), and



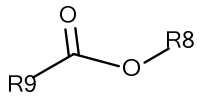
Formula (9)

wherein R7 is H or C1-6 alkyl and t is 1 to 6.

[0312] More preferably, the monomer is selected from the group consisting of 2-(methacryloyloxyethyl)-2'-(trimethylammoniumethyl) phosphate (HEMA-PC) and 2-(acryloyloxyethyl)-2'-(trimethylammoniumethyl) phosphate.

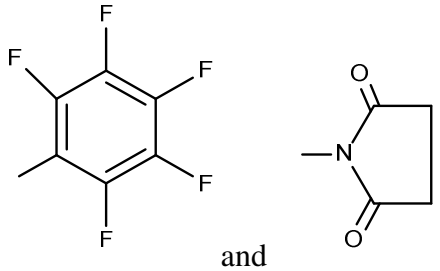
[0313] Most preferably, the monomer is 2-(methacryloyloxyethyl)-2'-(trimethylammoniumethyl) phosphate.

[0314] The second linker moiety preferably comprises an activated ester having the structure

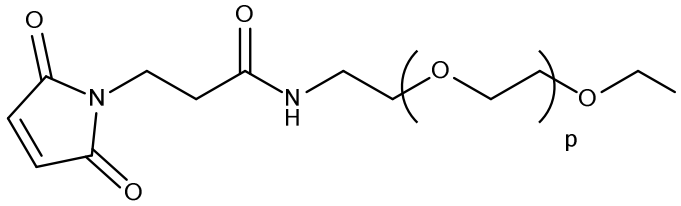


Formula (10)

wherein R8 is selected from the group consisting of



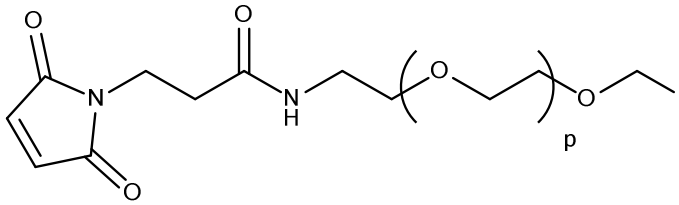
and R9 is



Formula (11)

wherein p is 1 to 12.

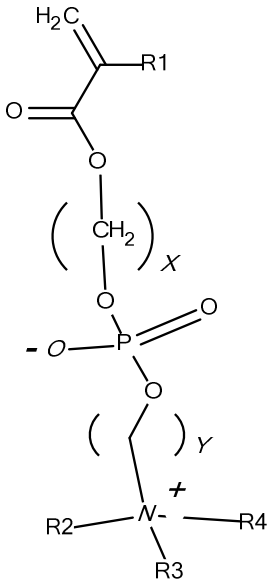
[0315] In more preferred embodiments of the present invention, the polymer has 9 arms, m is 2-4, R9 is



Formula (11)

wherein p is 4 to 15. Still more preferably, m is 4 and p is 12.

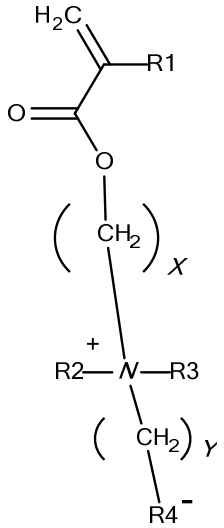
[0316] In some embodiments, the radically polymerizable monomer is



Formula (12)

wherein R1 is H or C1-6 alkyl, R2, R3, R4 are the same or different and are H or C1-4alkyl and X and Y are the same or different and are integers from 1-6. In some embodiments, R1, R2, R3 and R4 are each methyl and X and Y are each 2 in Formula (12).

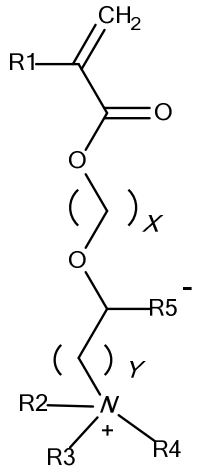
[0317] In some embodiments, the radically polymerizable monomer is



Formula (13)

wherein R1 is H or C1-6alkyl, R2 and R3 are the same or different and are H or C1-4alkyl, R4 is PO4-, SO3- or CO2- and X and Y are the same or different and are integers from 1-6. In some embodiments , R1, R2 and R3 are methyl, R4 is PO4- and X and Y are each 2 in Formula (13).

[0318] In some embodiments, the monomer is

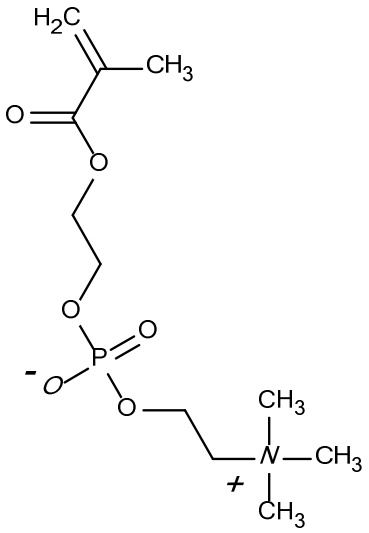


Formula (14)

wherein R1 is H or C1-6alkyl, R2, R3 and R4 are the same or different and are H or C1-4alkyl, R5 is PO4-, SO3- or CO2- and X and Y are the same or different and are integers from 1-6. In some embodiments, R1, R2, R3 and R4 are methyl, R5 is PO4- and X and Y are 2 in Formula (14).

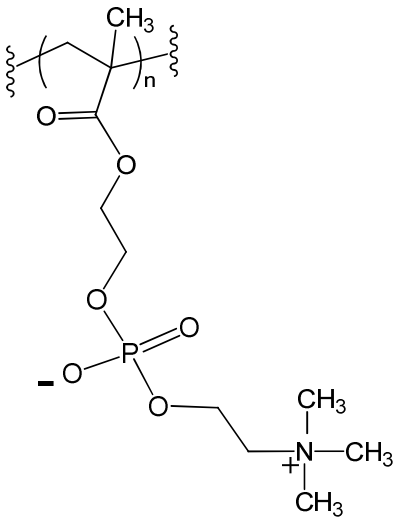
[0319] When a polymer is the to be conjugated via a cysteine (or other specified residue), the polymer can be linked directly or indirectly to the residue (e.g., with an intervening initiator, and or spacer or the like).

[0320] In some embodiments, the phosphorylcholine containing polymer comprises 2-(methacryloyloxyethyl)-2'-(trimethylammonium)ethyl phosphate (MPC) monomers as set forth below:



Formula (15),

such that the polymer comprises the following repeating units:



Formula (16);

where n is an integer from 1 to 3000 and the wavy lines indicate the points of attachment between monomer units in the polymer.

[0321] In some embodiments, the polymer has three or more arms, or is synthesized with an initiator comprising 3 or more polymer initiation sites. In some embodiments, the polymer has 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms, or is synthesized with an initiator comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 polymer initiation sites. More preferably, the polymer has 3, 6, or 9 arms, or is synthesized with an initiator comprising 3, 6, or 9 polymer initiation sites. In some embodiments, the polymer has 9 arms, or is synthesized with an initiator comprising 9 polymer initiation sites.

[0322] In some embodiments, the polymer that is added has a molecular weight between about 300,000 and about 1,750,000 Da (SEC-MALs). In some embodiments, the polymer has a molecular weight between about 500,000 and about 1,000,000 Da. In some embodiments, the polymer has a molecular weight of between about 600,000 to about 900,000 Da. In some embodiments, the polymer has a molecular weight of between about 750,000 to about 850,000 Da. In some embodiments, the polymer has a molecular weight of between about 800,000 to about 850,000 Da. In some embodiments, the polymer has a molecular weight of between about 750,000 to about 800,000 Da.

[0323] In some embodiments, any of the antibodies and/or Ab-Traps described herein can be further conjugated to a polymer to form a bioconjugate. The molecular weight of the bioconjugate (in total, SEC-MALs) can be between about 350,000 and 2,000,000 Daltons, for example, between about 450,000 and 1,900,000 Daltons, between about 550,000 and 1,800,000 Daltons, between about 650,000 and 1,700,000 Daltons, between about 750,000 and 1,600,000 Daltons, between about 850,000 and 1,500,000 Daltons, between about 900,000 and 1,400,000 Daltons, between about 950,000 and 1,300,000 Daltons, between about 900,000 and 1,000,000 Daltons, between about 1,000,000 and 1,300,000 Daltons, between about 850,000 and 1,300,000 Daltons, between about 850,000 and 1,000,000 Daltons, and between about 1,000,000 and 1,200,000 Daltons. In some embodiments, the bioconjugate has a molecular weight between about 350,000 and 1,900,000 Daltons.

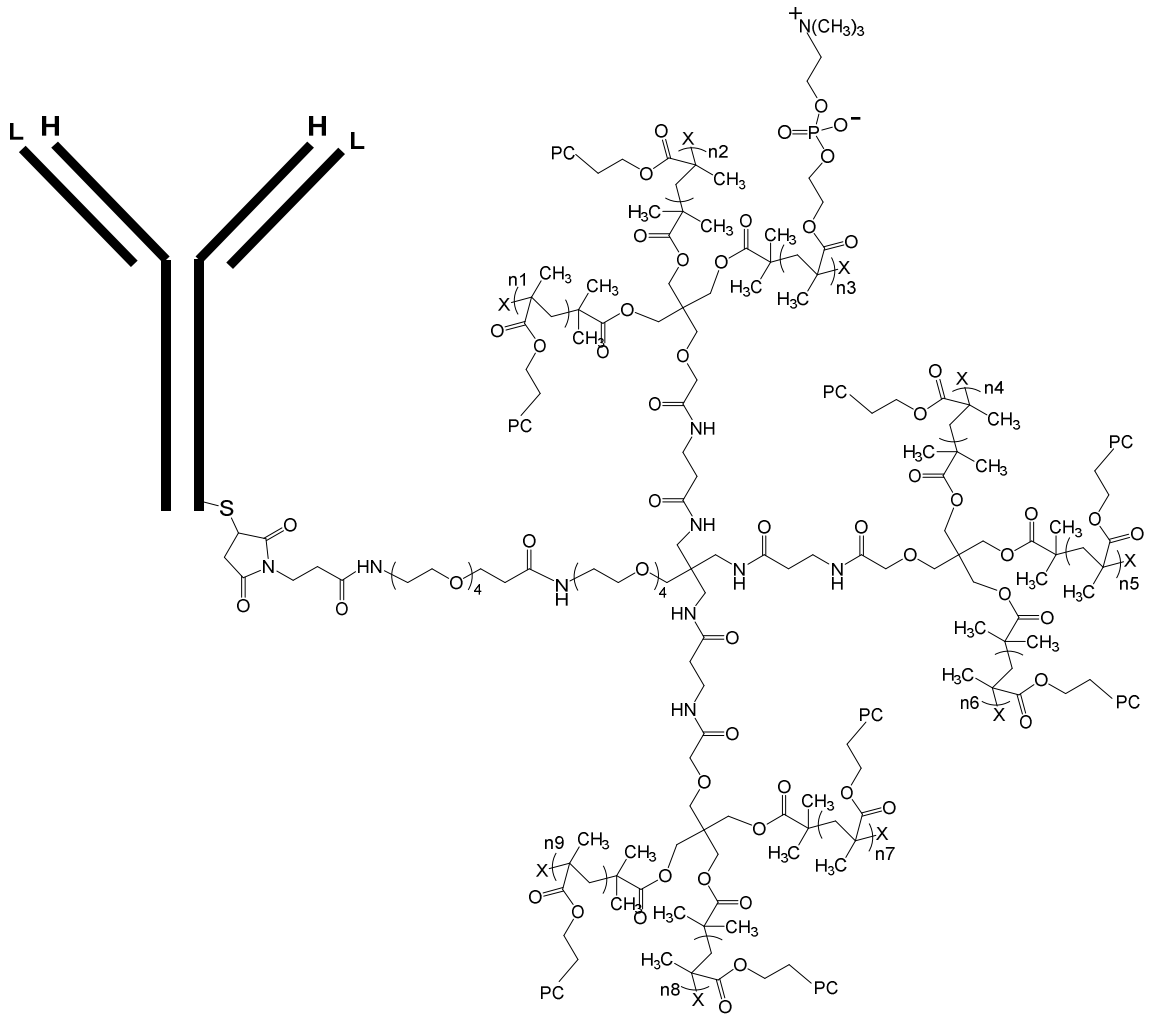
[0324] In some embodiments, the antibody and/or Ab-Trap conjugate is purified. In some embodiments, the polymer in aspect of the antibody and/or Ab-Trap conjugate is polydisperse, i.e. the polymer PDI is not 1.0. In some embodiments, the PDI is less than 1.5. In

some embodiments, the PDI is less than 1.4. In some embodiments, the PDI is less than 1.3. In some embodiments the PDI is less than 1.2. In some embodiments the PDI is less than 1.1. In some embodiments, the conjugate PDI is equal to or less than 1.5.

[0325] In some embodiments, the antibody and/or Ab-Trap conjugate has an anti-IL-6 immunoglobulin G (IgG) bonded to a polymer, which polymer comprises MPC monomers, wherein the sequence of the anti-IL-6 heavy chain is in Table 1A, and the sequence of the anti-IL-6 light chain is in Table 1B, and wherein the antibody and/or Ab-Trap is bonded only at C442 to the polymer. In some embodiments, the polymer has 9 arms and has a molecular weight of between about 600,000 to about 1,000,000 Da.

[0326] In some embodiments, the antibody and/or Ab-Trap conjugate has an anti-IL-6 immunoglobulin G (IgG) bonded to a polymer, which polymer comprises MPC monomers, wherein the sequence of the anti-IL-6 heavy chain comprises in Table 1A, and the sequence of the anti-IL-6 light chain comprises in Table 1B, and wherein the antibody is bonded only at C443 (EU numbering to the polymer. In some embodiments, the polymer has 9 arms and has a molecular weight of between about 600,000 to about 1,000,000 Da. In some embodiments, the conjugate comprises a polymer that has 9 arms and the polymer has a molecular weight of between about 600,000 to about 900,000 Da.

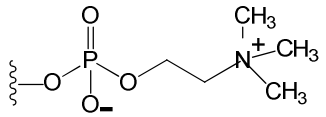
[0327] In some embodiments, the antibody conjugate has the following structure:



Formula (17)

wherein: each heavy chain of the anti-IL-6 antibody is denoted by the letter H, and each light chain of the anti-IL-6 antibody is denoted by the letter L (which, in some embodiments, can further be linked to the Ab-Trap arrangement, as shown in FIG. 6);

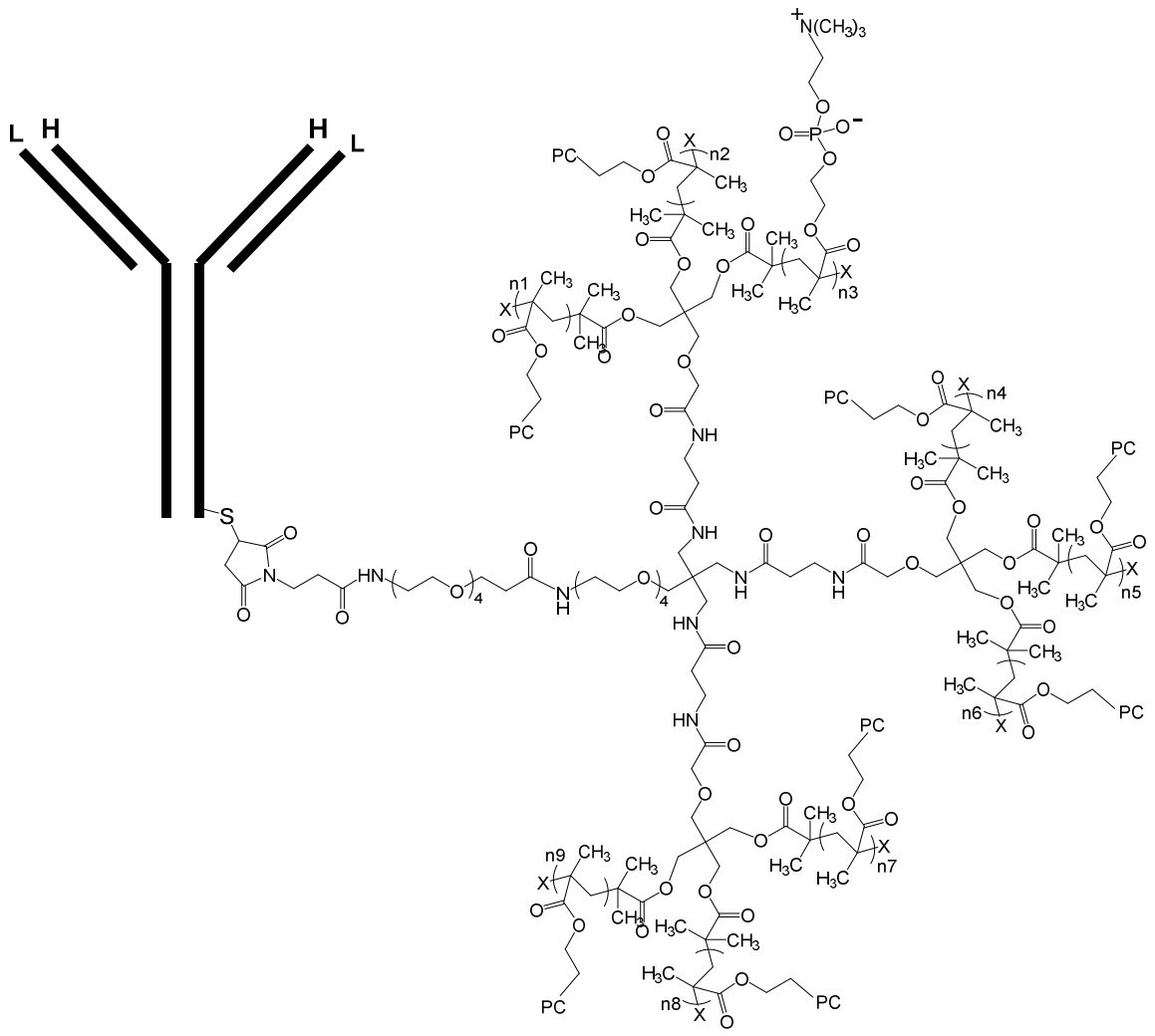
the polymer is bonded to the anti-IL-6 antibody (and/or Ab-Trap) through the sulfhydryl of C443 (EU numbering), which bond is depicted on one of the heavy chains; PC is,



where the curvy line indicates the point of attachment to the rest of the polymer; wherein X=a) OR where R=H, Methyl, ethyl, propyl, isopropyl, b) H, or c) any halide, including Br; and n1, n2, n3, n4, n5, n6, n7, n8 and n9 are the same or different such that the sum of n1, n2, n3, n4, n5, n6, n6,

n7, n8 and n9 is 2500 plus or minus 10%. In some embodiments, n1, n2, n3, n4, n5, n6, n7, n8 and n9 are the same or different and are integers from 0 to 3000. In some embodiments, n1, n2, n3, n4, n5, n6, n7, n8 and n9 are the same or different and are integers from 0 to 500. In some embodiments, X=OR, where R is a sugar, an aminoalkyl, mono-substituted, poly-substituted or unsubstituted variants of the following residues: saturated C_1 -C_{24} alkyl, unsaturated C_2 -C_{24} alkenyl or C_2 -C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxy carbonyl, alkoxy carbonylacyl, amino, aminocarbonyl, aminocarboyloxy, nitro, azido, phenyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl, --CO--O--R_7, carbonyl --CCO--R_7, --CO--NR_8R_9, --$(CH_2)_n$--$COOR_7$, --CO--$(CH)_n$--$COOR_7$, --$(CH_2)_n$--NR_8R_9, ester, alkoxycarbonyl, aryloxycarbonyl, wherein n is an integer from 1 to 6, wherein each R_7, R_8 and R_9 is separately selected from the group consisting of a hydrogen atom, halogen atom, mono-substituted, poly-substituted or unsubstituted variants of the following residues: saturated C_1- C_{24} alkyl, unsaturated C_2 -C_{24} alkenyl or C_2- C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxy carbonyl, alkoxy carbonylacyl, amino, aminocarbonyl, aminocarboyloxy, nitro, azido, phenyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl, a 5-membered ring, and a 6-membered ring. In some embodiments, Formula 17 can be part of a fusion protein, which would further include some or all of the sequence of Table 1C, so as to make an IL-6 Ab VEGF Trap fusion protein. In some embodiments, the fusion in FIG. 27 can be used within formula 17, with the fusion replacing the depicted antibody.

[0328] In some embodiments, the antibody conjugate has the following structure:

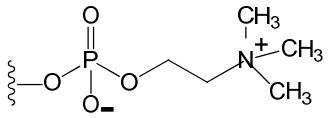


Formula (17)

wherein:

each heavy chain of the antibody is denoted by the letter H, and each light chain of the anti-IL-6 antibody is denoted by the letter L (which, in some embodiments, can further be linked to the Ab-Trap arrangement, as shown in FIG. 6);

the polymer is bonded to the antibody through the sulfhydryl of C443 (EU numbering), which bond is depicted on one of the heavy chains;

PC is  , where the curvy line indicates the point of attachment to the rest of the polymer, where X=a) OR where R=H, methyl, ethyl, propyl, isopropyl, b) H, or c) any halide, including Br; and

n1, n2, n3, n4, n5, n6, n7, n8 and n9 are the same or different such that the sum of n1, n2, n3, n4, n5, n6, n6, n7, n8 and n9 is 2500 plus or minus 15%. In some embodiments, the sum of n1, n2, n3, n4, n5, n6, n6, n7, n8 and n9 is about 1500 to about 3500 plus or minus about 10% to about 20%. In some embodiments, the fusion in FIG. 27 can be used within formula 17, with the fusion replacing the depicted antibody.

[0329] In some embodiments, the antibody and/or Ab-Trap conjugate is present in a liquid formulation. In some embodiments, the antibody and/or Ab-Trap conjugate is combined with a pharmaceutically acceptable carrier.

[0330] In some embodiments, the isotype of the anti-IL-6 antibody (and/or IL-6 Ab-VEGF Trap) heavy chain, is IgG1 and has a CH_1, hinge, CH_2 and CH_3 domains. In some embodiments the light chain isotype is kappa.

[0331] In some embodiments, the IgG1 domain of the anti-IL-6 antibody (and/or IL-6 Ab-VEGF Trap) has one or more mutations to modulate effector function, such as ADCC, ADCP, and CDC. In some embodiments, the IgG1 mutations reduce effector function. In some embodiments the amino acids to use for effector function mutations include (EU numbering) E233X, L234X, L235X, G236X, G237X, G236X, D270X, K322X, A327X, P329X, A330X, A330X, P331X, and P331X, in which X is any natural or non-natural amino acid. In some embodiments, the mutations include one or more of the following: E233P, L234V, L234A, L235A, G237A, A327G, A330S and P331S (EU numbering). In some embodiments, the anti-IL-6 heavy chain has the following mutations (EU numbering): L234A, L235A and G237A. In some embodiments, the number of effector function mutations relative to a natural human IgG1 sequence is no more than 10. In some embodiments the number of effector function mutations relative to a natural human IgG1 sequence is no more than 5, 4, 3, 2 or 1. In some embodiments, the antibody (and/or IL-6 Ab-VEGF Trap) has decreased Fc gamma binding and/or complement C1q binding, such that the antibody's ability to result in an effector function is decreased. This can be especially advantageous for ophthalmic indications/disorders.

[0332] In some embodiments, the anti-IL-6 antibody (and/or IL-6 Ab-VEGF Trap) comprises one or more of the following amino acid mutations: L234A, L235A, G237A, and L443C (EU numbering, or 451A, 452A, and 454A, and 660C in SEQ ID NO: 170, as shown in double underlining in FIG. 27).

[0333] In some embodiments, the anti-IL-6 antibody (and/or IL-6 Ab VEGFTrap) is or is part of a human immunoglobulin G (IgG1).

[0334] In some embodiments, the IL-6 antibody (and/or IL-6 Ab-VEGF Trap) comprises a heavy chain constant domain that comprises one or more mutations that reduce an immune-mediated effector function.

[0335] In some embodiments, the anti-IL-6 heavy chain has a cysteine residue added as a mutation by recombinant DNA technology which can be used to conjugate a half-life extending moiety. In some embodiments, the mutation is Q347C (EU numbering) and/or L443C (EU numbering). In some embodiments, the mutation is L443C (EU numbering). In some embodiments, the stoichiometry of antibody to polymer is 1:1; in other words, a conjugate has one molecule of antibody conjugated to one molecule of polymer.

[0336] The half-life of the anti-IL-6 antibodies can be extended by attachment of a "half-life ("half life") extending moieties" or "half-life ("half life") extending groups". Half-life extending moieties include peptides and proteins which can be expressed in frame with the biological drug of issue (or conjugated chemically depending on the situation) and various polymers which can be attached or conjugated to one or more amino acid side chain or end functionalities such as -SH, -OH, -COOH, -CONH2, -NH2, or one or more N- and/or O-glycan structures. Half-life extending moieties generally act to increase the *in vivo* circulatory half-life of biologic drugs.

[0337] Examples of peptide/protein half-life extending moieties include Fc fusion (Capon DJ, Chamow SM, Mordenti J, et al. Designing CD4 immunoadhesions for AIDS therapy. Nature. 1989. 337:525-31), human serum albumin (HAS) fusion (Yeh P, Landais D, Lemaitre M, et al. Design of yeast-secreted albumin derivatives for human therapy: biological and antiviral properties of a serum albumin-CD4 genetic conjugate. Proc Natl Acad Sci USA. 1992. 89:1904-08), carboxy terminal peptide (CTP) fusion (Fares FA, Suganuma N. Nishimori K, et al. Design of a long-acting follitropin agonist by fusing the C-terminal sequence of the chorionic gonadotropin beta subunit to the follitropin beta subunit. Proc Natl Acad Sci USA. 1992. 89:4304-

08), genetic fusion of non-exact repeat peptide sequence (XTEN) fusion (Schellenberger V, Wang CW, Geething NC, et al. A recombinant polypeptide extends the in vivo half-life of peptides and proteins in a tunable manner. Nat Biotechnol. 2009. 27:1186-90), elastin like peptide (ELPylation) (MCpherson DT, Morrow C, Minehan DS, et al. Production and purification of a recombinant elastomeric polypeptide, G(VPGVG19-VPGV, from *Escheriachia coli*. Biotechnol Prog. 1992. 8:347-52), human transferrin fusion (Prior CP, Lai C-H, Sadehghi H et al. Modified transferrin fusion proteins. Patent WO2004/020405. 2004), proline-alanine-serine (PASylation) (Skerra A, Theobald I, Schlapsky M. Biological active proteins having increased in vivo and/or vitro stability. Patent WO2008/155134 A1. 2008), homo-amino acid polymer (HAPylation) (Schlapschy M, Theobald I, Mack H, et al. Fusion of a recombinant antibody fragment with a homo-amino acid polymer: effects on biophysical properties and prolonged plasma half-life. Protein Eng Des Sel. 2007. 20:273-84) and gelatin like protein (GLK) fusion (Huang Y-S, Wen X-F, Zaro JL, et al. Engineering a pharmacologically superior form of granulocyte-colony-stimulating-factor by fusion with gelatin-like protein polymer. Eur J. Pharm Biopharm. 2010. 72:435-41).

[0338] Examples of polymer half-life extending moieties include polyethylene glycol (PEG), branched PEG, PolyPEG® (Warwick Effect Polymers; Coventry, UK), polysialic acid (PSA), starch, hydroxylethyl starch (HES), hydroxyalkyl starch (HAS), carbohydrate, polysaccharides, pullulane, chitosan, hyaluronic acid, chondroitin sulfate, dermatan sulfate, dextran, carboxymethyl-dextran, polyalkylene oxide (PAO), polyalkylene glycol (PAG), polypropylene glycol (PPG), polyoxazoline, polyacryloylmorpholine, polyvinyl alcohol (PVA), polycarboxylate, polyvinylpyrrolidone, polyphosphazene, polyoxazoline, polyethylene-co-maleic acid anyhydride, polystyrene-co-maleic acid anhydride, poly(1-hydroxymethyethylene hydroxymethylformal) (PHF), a zwitterionic polymer, a phosphorylcholine containing polymer and a polymer comprising MPC, Poly (Gly_x-Ser_y), Hyaluronic acid (HA), Heparosan polymers (HEP), Fleximers, Dextran, and Poly-sialic acids (PSA).

[0339] In one embodiment a half-life extending moiety can be conjugated to an antibody via free amino groups of the protein using N-hydroxysuccinimide (NHS) esters. Reagents targeting conjugation to amine groups can randomly react to ϵ-amine group of lysines, α-amine group of N-terminal amino acids, and δ-amine group of histidines.

[0340] However, the anti-IL-6 antibodies (and/or IL-6 Ab-VEGF Trap) disclosed herein can have many amine groups available for polymer conjugation. Conjugation of polymers

to free amino groups, thus, might negatively impact the ability of the antibody proteins to bind to IL-6 (and/or IL-6 Ab-VEGF Trap).

[0341] In some embodiments, a half-life extending moiety is coupled to one or more free SH groups using any appropriate thiol-reactive chemistry including, without limitation, maleimide chemistry, or the coupling of polymer hydrazides or polymer amines to carbohydrate moieties of the antibody after prior oxidation. In some embodiments maleimide coupling is used In some embodiments, coupling occurs at cysteines naturally present or introduced via genetic engineering.

[0342] In some embodiments, conjugates of antibody and high MW polymers serving as half-life extenders are provided. In some embodiments, a conjugate comprises an antibody (and/or IL-6 Ab-VEGF Trap) that is coupled to a zwitterionic polymer wherein the polymer is formed from one or more monomer units and wherein at least one monomer unit has a zwitterionic group is provided. In some embodiments, the zwitterionic group is phosphorylcholine.

[0343] In some embodiments, one of the monomer units is HEMA-PC. In some embodiments, a polymer is synthesized from a single monomer which is HEMA-PC.

[0344] In some embodiments, some antibody (and/or IL-6 Ab VEGFTrap) conjugates have 2, 3, or more polymer arms wherein the monomer is HEMA-PC. In some embodiments, the conjugates have 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 polymer arms wherein the monomer is HEMA-PC. In some embodiments, the conjugates have 3, 6 or 9 arms. In some embodiments, the conjugate has 9 arms.

[0345] In some embodiments, polymer-antibody (and/or polymer-IL-6 Ab-VEGFTrap) conjugates have a polymer portion with a molecular weight of between 100,000 and 1,500,000 Da. In some embodiments, the conjugate has a polymer portion with a molecular weight between 500,000 and 1,000,000 Da. In some embodiments, the conjugate has a polymer portion with a molecular weight between 600,000 to 800,000 Da. In some embodiments, the conjugate has a polymer portion with a molecular weight between 600,000 and 850,000 Da and has 9 arms. When a molecular weight is given for an antibody conjugated to a polymer, the molecular weight will be the addition of the molecular weight of the protein, including any carbohydrate moieties associated therewith, and the molecular weight of the polymer.

[0346] In some embodiments, an anti-IL-6 antibody (and/or IL-6 Ab VEGF Trap) has a HEMA-PC polymer which has a molecular weight measured by Mw of between about 100 kDa

and 1650 kDa is provided. In some embodiments, the molecular weight of the polymer as measured by Mw is between about 500 kDa and 1000 kDa. In some embodiments, the molecular weight of the polymer as measured by Mw is between about 600 kDa to about 900 kDa. In some embodiments, the polymer molecular weight as measured by Mw is 750 or 800 kDa plus or minus 15%.

[0347] In some embodiments, the polymer is made from an initiator suitable for ATRP having one or more polymer initiation sites. In some embodiments, the polymer initiation site has a 2-bromoisobutyrate site. In some embodiments, the initiator has 3 or more polymer initiation sites. In some embodiments, the initiator has 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 polymer initiation sites. In some embodiments, the initiator has 3, 6 or 9 polymer initiation sites. In some embodiments, the initiator has 9 polymer initiation sites. In some embodiments, the initiator is OG1786.

[0348] The anti-IL-6 antibodies (and/or IL-6 Ab-VEGFTrap) can be produced by recombinant expression including (i) the production of recombinant DNA by genetic engineering, (ii) introducing recombinant DNA into prokaryotic or eukaryotic cells by, for example and without limitation, transfection, electroporation or microinjection, (iii) cultivating the transformed cells, (iv) expressing antibody, *e.g.* constitutively or on induction, and (v) isolating the antibody, *e.g.* from the culture medium or by harvesting the transformed cells, in order to (vi) obtain purified antibody.

[0349] The anti-IL-6 antibodies (and/or IL-6 Ab-VEGF Trap) can be produced by expression in a suitable prokaryotic or eukaryotic host system characterized by producing a pharmacologically acceptable antibody molecule. Examples of eukaryotic cells are mammalian cells, such as CHO, COS, HEK 293, BHK, SK-Hip, and HepG2. Other suitable expression systems are prokaryotic (e.g., E. coli with pET/BL21 expression system), yeast (Saccharomyces cerevisiae and/or Pichia pastoris systems), and insect cells.

[0350] A wide variety of vectors can be used for the preparation of the antibodies (and/or IL-6 Ab-VEGF Trap) disclosed herein and are selected from eukaryotic and prokaryotic expression vectors. Examples of vectors for prokaryotic expression include plasmids such as, and without limitation, preset, pet, and pad, wherein the promoters used in prokaryotic expression vectors include one or more of, and without limitation, lac, trc, trp, recA, or araBAD. Examples of vectors for eukaryotic expression include: (i) for expression in yeast, vectors such as, and

without limitation, pAO, pPIC, pYES, or pMET, using promoters such as, and without limitation, AOX1, GAP, GAL1, or AUG1; (ii) for expression in insect cells, vectors such as and without limitation, pMT, pAc5, pIB, pMIB, or pBAC, using promoters such as and without limitation PH, p10, MT, Ac5, OpIE2, gp64, or polh, and (iii) for expression in mammalian cells, vectors such as, and without limitation, pSVL, pCMV, pRc/RSV, pcDNA3, or pBPV, and vectors derived from, in one aspect, viral systems such as and without limitation vaccinia virus, adeno-associated viruses, herpes viruses, or retroviruses, using promoters such as and without limitation CMV, SV40, EF-1, UbC, RSV, ADV, BPV, and beta-actin.

[0351] In some embodiments, there is at least one polymer per protein. In some embodiments, the ratio of protein to polymer is 1:4 to 1:6. In some embodiments, the ratio of protein (Ab) to polymer is 1:5 molar ratio. In some embodiments, the amount of protein is 5, 4, 3, 2, or about 2.4 mg/ml.

[0352] In some embodiments, the purification following the combination of the polymer and the antibody further comprises a cation exchange column.

[0353] In some embodiments, the presence of the polymer attached to the IL-6 antagonist antibodies and/or IL-6 Ab VEGF Traps can provide a deeper potency due to the presence of the polymer itself. In some embodiments, the presence of the polymer, when used in combination with a heparin binding domain, (such as in VEGF) results in a conjugated molecule with deeper potency. In some embodiments, as shown in the HUVECs data provided in the examples below, the molecule is superior in angiogenesis. In some embodiments, this is a difference in nature of the conjugate molecule, not simply a difference in degree (e.g., comparing the conjugate to the unconjugated molecules). Furthermore, as shown in the HUVEC proliferation assays in the examples below, in some embodiments, the presence of the polymer provides for synergistic inhibition.

[0354] In addition, in some embodiments, as cell densities are increased (e.g., from 4,000 to 6,000), the potency of the conjugate may also improve.

[0355] In some embodiments, the amount (by weight of protein) of the conjugate used is more than 50 mg/ml. It can be, for example, 55, 60, 65, 70 or greater mg/ml (by weight of pre-conjugation protein).

Method of Conjugating Proteins to Polymers

[0356] In some embodiments, a method is presented of preparing a therapeutic protein-half life extending moiety conjugate having the step of conjugating a therapeutic protein which has a cysteine residue added via recombinant DNA technology to a half-life extending moiety having a sulfhydryl specific reacting group selected from the group consisting of maleimide, vinylsulfones, orthopyridyl-disulfides, and iodoacetamides to provide the therapeutic protein-half life extending moiety conjugate.

[0357] In some embodiments a method of preparing the antibody (and/or IL-6 Ab-VEGF Trap) conjugate is provided. As shown in FIG. 10, the method comprises reducing the protein with a 30x molar excess of the TCEP reducing agent (FIG. 10). After reduction, the antibody is oxidized to produce a decapped antibody where the inter- and intra- light and heavy chain disulfide bonds naturally occurring in the antibody are formed, but the engineered Cysteine on the heavy chain position L443C (EU numbering) remains to be decapped (FIG. 10). The antibody is then conjugated by adding an excipient and adding 2-10x molar excess of a maleimide biopolymer. (FIG. 10). The biopolymer links to the antibody through a covalent thiolether linkage (FIG. 10). After conjugation, the antibody conjugate is purified with both unconjugated antibody and polymer removed (FIG. 10). In embodiments in which the protein is an Ab-Trap, the same position on the antibody can be used and the same approach employed.

[0358] The protein and process described above can be varied as well. Thus, in some embodiments, a process for preparing a conjugated protein (which need not be an antibody or an anti-IL-6 antibody) is provided. The process includes reducing one or more cysteines in a protein to form a decapped protein in a solution. After reducing the one or more cysteines the decapped protein is reoxidized to restore at least one disulfide linkage in the reduced protein while ensuring that an engineered cysteine residue in the protein remains in a free thiol form to form a reoxidized decapped protein in the solution. At least one excipient is then added to the solution. The excipient reduces a polymer induced protein precipitation. After the excipient is added, a polymer is added to the solution, which is conjugated to the reoxidized decapped protein at the engineered cysteine residue to form a conjugated protein.

[0359] In some embodiments, the molar excess of the reducing agent can be altered to any amount that functions. In some embodiments 10, 20, 30, 40, 50, 60, 70, 80, 90x molar excess of the reducing agent (which need not be TCEP in all embodiments) can be employed. In some

embodiments, the reducing agent can be Trisodium 3,3',3''-phosphinetriyltris(benzene-1-sulphonate) (TPPTS).In some embodiments, any antibody (therapeutic or otherwise) can be employed. In some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15x molar excess of a maleimide biopolymer can be employed. In some embodiments, there is an excess of decapped protein to polymer. In some embodiments, the amount of the reduced protein is less than the amount of the polymer. In some embodiments, the amount of the reduced protein is 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, 1% of the amount of the polymer. In some embodiments, 10-15 times as much polymer is used as protein. In some embodiments the amount of the reduced antibody is greater than the amount of the polymer. In some embodiments the amount of the polymer is greater than the amount of the reduced antibody (and/or IL-6 Ab-VEGF Trap).

[0360] In some embodiments, the purification step is optional.

[0361] In some embodiments, the method of making an antibody conjugate (and/or Ab-Trap) comprises conjugating an anti-IL-6 antibody (and/or IL-6 Ab-VEGF Trap) to a phosphorylcholine containing polymer. In some embodiments the method comprises the steps of conjugating an anti-IL-6 antibody to a phosphorylcholine containing polymer. The anti-IL-6 antibody comprises an amino residue added via recombinant DNA technology. In some embodiments, the added amino acid residue is a cysteine residue. In some embodiments, the cysteine residue is added outside a variable region of the antibody. The cysteine residue can be added to either the heavy chain or light chain of the antibody.

[0362] In some embodiments, the polymer comprises or consists of a phosphorylcholine containing polymer. In some embodiments, the phosphorylcholine containing polymer comprises a sulfhydryl specific reacting group selected from the group consisting of a maleimide, a vinylsulfone, an orthopyridyl-disulfide, and an iodoacetamide. In some embodiments, the sulfhydryl specific reacting group on the phosphorylcholine containing polymer reacts with the cysteine residue on the anti-IL-6 antibody to make the antibody (and/or IL-6 Ab VEGF Trap) conjugate.

[0363] In some embodiments, the protein to be conjugated can be an antibody, an antibody protein fusion (e.g. IL-6 Ab-VEGF Trap), or a binding fragment thereof. In some embodiments, the protein is not an antibody but is an enzyme, a ligand, a receptor, or other protein

or mutants or variants thereof. In some embodiments, the native protein contains at least one disulfide bond and at least one non-native cysteine.

[0364] In some embodiments, the excipient can be an acid or a base. In some embodiments, the excipient is a detergent, a sugar, or a charged amino acid. In some embodiments, the excipient assists in keeping the protein in solution during the conjugation to the polymer. In some embodiments, the excipient is added to the solution containing the protein, prior to the addition of the polymer to the solution that contains the protein.

[0365] In some embodiments, the reaction occurs under aqueous conditions between about pH 5 to about pH 9. In some embodiments, the reaction occurs between 6.0 and 8.5, between 6.5 and 8.0 or between 7.0 and 7.5.

[0366] In some embodiments, the polymer is conjugated to the protein at 2-37 degrees Celsius. In some embodiments, the conjugation occurs at 0-40 degrees Celsius, 5-35 degrees Celsius, 10-30 degrees Celsius, and 15-25 degrees Celsius.

[0367] In some embodiments, the conjugated proteins described herein can be contacted to an ion exchange medium or hydrophobic interaction chromatography or affinity chromatography medium for purification (to remove the conjugated from the unconjugated). In some embodiments, the ion exchange medium, hydrophobic interaction chromatography, and/or affinity chromatography medium separates the conjugated protein from the unconjugated free polymer and from the unconjugated reoxidized decapped protein.

[0368] In some embodiments, the processes described herein and outlined in FIG. 10 involves an excipient that is capable of facilitating and/or maintaining a solubility system. In some embodiments, the process allows the solution to maintain the solubility of the two components meant to interact. This can include the solubility of the protein and the polymer and then the end conjugate as well. In some embodiments, without the excipient approach, the issue can be that while the protein it is soluble, when the biopolymer is added, the solubility of the solution (e.g., protein) drops and it crashes/precipitates out of solution. Of course, when the protein crashes out, it is not available to conjugate efficiently with the biopolymer. Thus, an excipient can be employed to maintain the solubility of the protein in the presence of the biopolymer so the two can couple to form the protein conjugate (or as depicted in FIG. 10, an antibody conjugate). This also allows for the solubility of the conjugate to be maintained.

[0369] In some embodiments, the polymers disclosed herein can comprise one or more of the following: a zwitterion, a phosphorylcholine, or a PEG linker bridging a center of a polymer branching point to the maleimide functional group. In some embodiments, any of the polymers provided herein can be added to a protein via the methods provided herein.

[0370] In some embodiments, any of the proteins provided herein can be conjugated to any of the polymers provided herein via one or more of the methods provided herein.

[0371] In some embodiments, the process(es) provided herein allow(s) for larger scale processing to make and purify protein and/or antibody conjugates. In some embodiments, the volume employed is at least 1 liter, for example 1, 10, 100, 1,000, 5,000, 10,000, liters or more. In some embodiments, the amount of the antibody conjugate produced and/or purified can be 0.1, 1, 10, 100, 1000, or more grams.

[0372] In some embodiments, the therapeutic protein may be any of the anti-IL-6 antibodies (and/or IL-6 Ab-VEGF Traps) described herein having a cysteine residue added via recombinant DNA technology. In some embodiments, the anti-IL-6 antibody heavy chain has the following CDRs: CDR_H1: in Table 1A, CDR_H2: in Table 1A, and CDR_H3: in Table 1A. The heavy chain can also have arginine (R) at position 84 (via sequential numbering). In some embodiments, the anti-IL-6 light chain has the following CDRs: CDR_L1: in Table 1B, CDR_L2: in Table 1B, and CDR_L3: in Table 1B.

[0373] In some embodiments, the anti-IL-6 antibody (and/or IL-6 Ab-VEGF Trap) includes IgG1. In some embodiments, the heavy chain has one or more mutations to modulate effector function. In some embodiments, the mutations are to one or more of the following amino acid positions (EU numbering): E233, L234, L235, G236, G237, A327, A330, and P331. In some embodiments, the mutations are selected from the group consisting of: E233P, L234V, L234A, L235A, G237A, A327G, A330S and P331S (EU numbering). In some embodiments, the mutations are (EU numbering) L234A, L235A and G237A.

[0374] In some embodiments, the cysteine residue added to the therapeutic protein via recombinant DNA technology should not be involved in Cys-Cys disulfide bond pairing. In this regard, therapeutic proteins may be dimeric. So for example, an intact anti-IL-6 antibody (and/or IL-6 Ab-VEGF Trap) has two light chains and two heavy chains. If a Cys residue is introduced into the heavy chain for instance, the intact antibody will have two such introduced cysteines at identical positions and the possibility exists that these cysteine residues will form intra-chain

disulfide bonds. If the introduced cysteine residues form Cys-Cys disulfide bonds or have a propensity to do so, that introduced Cys residue will not be useful for conjugation. It is known in the art how to avoid positions in the heavy and light chains that will give rise to intra-chain disulfide pairing. See, e.g., U.S. Patent Application No. 2015/0158952.

[0375] In some embodiments, the cysteine residue introduced via recombinant DNA technology is selected from the group consisting of (EU numbering) Q347C and L443C. In some embodiments, the cysteine residue is L443C (EU numbering). In some embodiments, the heavy chain the antibody has the amino acid sequence set forth in Table 1A and the light chain has the amino acid sequence of Table 1B.

[0376] In some embodiments, the sulfhydral specific reacting group is maleimide.

[0377] In some embodiments, the half-life extending moiety is selected from the group consisting of polyethylene glycol (PEG), branched PEG, PolyPEG® (Warwick Effect Polymers; Coventry, UK), polysialic acid (PSA), starch, hydroxylethyl starch (HES), hydroxyalkyl starch (HAS), carbohydrate, polysaccharides, pullulane, chitosan, hyaluronic acid, chondroitin sulfate, dermatan sulfate, dextran, carboxymethyl-dextran, polyalkylene oxide (PAO), polyalkylene glycol (PAG), polypropylene glycol (PPG), polyoxazoline, polyacryloylmorpholine, polyvinyl alcohol (PVA), polycarboxylate, polyvinylpyrrolidone, polyphosphazene, polyoxazoline, polyethylene-co-maleic acid anyhydride, polystyrene-co-maleic acid anhydride, poly(1-hydroxymethyethylene hydroxymethylformal) (PHF), a zwitterionic polymer, a phosphorylcholine containing polymer and a polymer comprising 2-methacryloyloxy-2'-ethyltrimethylammoniumphosphate (MPC).

[0378] In some embodiments, the half-life extending moiety is a zwitterionic polymer. In some embodiments, the zwitterion is phosphorylcholine, i.e. a phosphorylcholine containing polymer. In some embodiments, the polymer is composed of MPC units.

[0379] In some embodiments, the MPC polymer has three or more arms. In some embodiments, the MPC polymer has 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms. In some embodiments, the MPC polymer has 3, 6, or 9 arms. In some embodiments, the MPC polymer has 9 arms. In some embodiments, the polymer is synthesized with an initiator comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more polymer initiation sites

[0380] In some embodiments, the MPC polymer has a molecular weight between about 300,000 and 1,750,000 Da. In some embodiments, the MPC polymer has a molecular weight between about 500,000 and 1,000,000 Da or between about 600,000 to 900,000 Da.

[0381] In some embodiments, the method of preparing a therapeutic protein-half life extending moiety conjugate has an additional step of contacting the therapeutic protein with a thiol reductant under conditions that produce a reduced cysteine sulfhydryl group. As discussed above, it is preferable that the cysteine residue added via recombinant DNA technology are unpaired, i.e. are not involved in Cys-Cys intra chain disulfide bonds or are not substantially involved in such bonding. However, Cys residues which are not involved in such Cys-Cys disulfide bonding and are free for conjugation are known to react with free cysteine in the culture media to form disulfide adducts. See, e.g., WO 2009/052249. A cysteine so derivatized will not be available for conjugation. To free the newly added cysteine from the disulfide adduct, the protein after purification is treated with a reducing agent, e.g., dithiothreitol. However, such treatment with a reducing agent will reduce all of the cysteine residues in the therapeutic protein, including native cysteines many of which are involved in inter and intra chain Cys-Cys disulfides bonds. The native Cys-Cys disulfides are generally crucial to protein stability and activity and they should be reformed. In some embodiments, all native (e.g., inter and intra) Cys-Cys disulfides are reformed.

[0382] To reform native inter and intra-chain disulfide residues, after reduction to remove the cysteine disulfide adducts, the therapeutic protein is exposed to oxidizing conditions and/or oxidizing agents for a prescribed period of time, e.g., overnight. In some embodiments, ambient air exposure overnight can be used to achieve reformation of the native disulfide bonds. In some embodiments, an oxidizing agent is employed to restore the native disulfides. In some embodiments, the oxidizing agent is selected from the group consisting of aqueous CuSO4 and dehydroascorbic acid (DHAA). In some embodiments, the oxidizing agent is DHAA. In some embodiments, the range of DHAA used is in the range of 5-30 equivalents. In some embodiments, the range is 10-30 equivalents. In some embodiments, the range is 15 equivalents.

[0383] In some embodiments, the thiol reductant is selected from the group consisting of: 3,3′,3″-Phosphanetriyltripropanoic acid (TCEP), dithiothreitol (DTT), dithioerythritol (DTE), sodium borohydride ($NaBH_4$), sodium cyanoborohydride (NaCNBH3), β-mercaptoethanol (BME), cysteine hydrochloride, Trisodium 3,3',3"-phosphinetriyltris(benzene-1-sulphonate) (TPPTS).and cysteine. In some embodiments, the thiol reductant is TCEP.

[0384] In some embodiments, the thiol reductant concentration is between 1 and 100 fold molar excess relative to the therapeutic protein concentration. In some embodiments, the thiol reductant concentration is between 20 to 50 fold molar excess relative to the therapeutic

protein concentration. In some embodiments, the thiol reductant is removed following incubation with the therapeutic protein prior to oxidation of the therapeutic protein.

[0385] In some embodiments, the method for conjugating a therapeutic protein to a half-life extending moiety has a further step of purifying the therapeutic protein conjugate after conjugation. In some embodiments, the therapeutic protein conjugate is purified using a technique selected from the group consisting of ion exchange chromatography, hydrophobic interaction chromatography, size exclusion chromatography, and affinity chromatography or combinations thereof.

[0386] In some embodiments, the therapeutic protein conjugate retains at least 20% biological activity relative to unconjugated therapeutic protein. In some embodiments, the therapeutic protein conjugate retains at least 50% biological activity relative to unconjugated therapeutic protein. In some embodiments, the therapeutic protein conjugate retains at least 90% biological activity relative to native therapeutic protein.

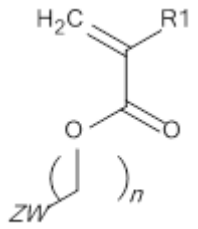
[0387] In some embodiments, the therapeutic protein conjugate has an increased half-life relative to unconjugated therapeutic protein. In some embodiments, the therapeutic protein conjugate has at least a 1.5 fold increase in half-life relative to unconjugated therapeutic protein. In some embodiments, the therapeutic protein conjugate has at least a 1.5, 2, 2.5, 3, 3.5, 4, 4.5 or 5 fold increase in half-life relative to unconjugated therapeutic protein.

[0388] In some embodiments, the zwitterionic polymer of the method of conjugating a therapeutic protein to a half-life extending moiety is a radically polymerizable monomer having a zwitterionc group and the method has a further step of polymerizing the free radically polymerizable zwitterionic monomer in a polymerization medium to provide a polymer, the medium comprising: the radically polymerizable zwitterionic monomer; a transition metal catalyst $M_t^{(q-1)+}$ wherein M_t is a transition metal, q is a higher oxidation state of the metal and q-1 is a lower oxidation state of the metal, wherein the metal catalyst is supplied as a salt of the form $Mt^{(q-1)+}X'_{(q-1)}$ wherein X' is a counterion or group or the transition metal catalyst is supplied in situ by providing the inactive metal salt at its higher oxidation state $M_t^{q+}X'_q$ together with a reducing agent that is capable of reducing the transition metal from the oxidized inactive state to the reduced active state; a ligand; and an initiator.

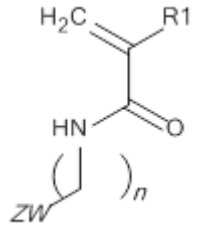
[0389] To function as an ATRP transition metal catalyst, the transition metal should have at least two readily accessible oxidation states separated by one electron, a higher oxidation

state and a lower oxidation state. In ATRP, a reversible redox reaction results in the transition metal catalyst cycling between the higher oxidation state and the lower oxidation state while the polymer chains cycle between having propagating chain ends and dormant chain ends. See, e.g., U.S. Patent No. 7,893,173.

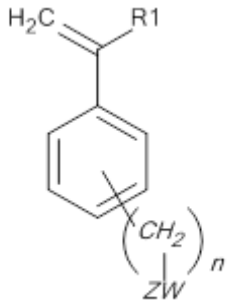
[0390] In some embodiments, the radically polymerizable zwitterionic monomer is selected from the group consisting of



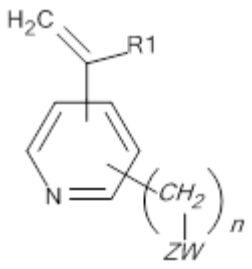
Formula (18)



Formula (19)



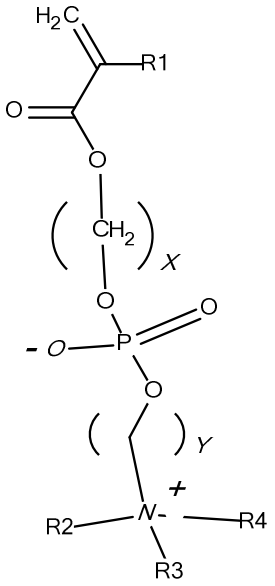
Formula (20), and



Formula (21)

wherein R1 is H or C1-6 alkyl, ZW is a zwitterion and n is an integer from 1-6.

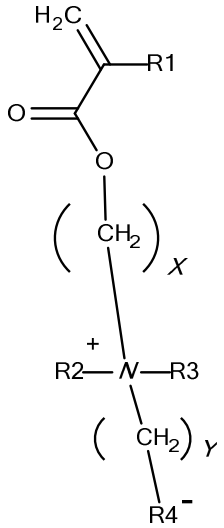
[0391] In some embodiments, the radically polymerizable monomer is



Formula (12)

wherein R1 is H or C1-6 alkyl, R2, R3, R4 are the same or different and are H or C1-4alkyl and X and Y are the same or different and are integers from 1-6. In some embodiments, R1, R2, R3 and R4 are each methyl and X and Y are each 2 in Formula (12).

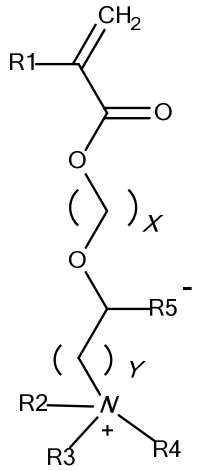
[0392] In some embodiments, the radically polymerizable monomer is



Formula (13)

wherein R1 is H or C1-6alkyl, R2 and R3 are the same or different and are H or C1-4alkyl, R4 is PO4-, SO3- or CO2- and X and Y are the same or different and are integers from 1-6. In some embodiments, R1, R2 and R3 are methyl, R4 is PO4- and X and Y are each 2 in Formula (13).

[0393] In some embodiments, the monomer is



Formula (14)

wherein R1 is H or C1-6alkyl, R2, R3 and R4 are the same or different and are H or C1-4alkyl, R5 is PO4-, SO3- or CO2- and X and Y are the same or different and are integers from 1-6. In some embodiments, R1, R2, R3 and R4 are methyl, R5 is PO4- and X and Y are 2 in Formula (14).

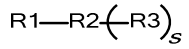
[0394] In some embodiments, the transition metal Mt is selected from the group consisting of Cu, Fe, Ru, Cr, Mo, W, Mn, Rh, Re, Co, V, Zn, Au, and Ag. In some embodiments, the metal catalyst is supplied as a salt of the form $Mt^{(q-1)+}X'_{(q-1)}$. $M_t^{(q-1)+}$ is selected from the group consisting of Cu^{1+}, Fe^{2+}, Ru^{2+}, Cr^{2+}, Mo^{2+}, W^{2+}, Mn^{3+}, Rh^{3+}, Re^{2+}, Co^{+}, V^{2+}, Zn^{+}, Au^{+}, and Ag^{+} and X' is selected from the group consisting of halogen, C_{1-6} alkoxy, $(SO_4)_{1/2}$, $(PO_4)_{1/3}$, $(R7PO_4)_{1/2}$, $(R7_2PO_4)$, triflate, hexaluorophosphate, methanesulfonate, arylsulfonate, CN and $R7CO_2$, where R7 is H or a straight or branched C_{1-6} alkyl group which may be substituted from 1 to 5 times with a halogen. In some embodiments, $M_t^{(q-1)+}$ is Cu^{1+} and X' is Br.

[0395] In some embodiments, $M_t^{(q-1)+}$ is supplied in situ. In some embodiments, $M_t^{q+}X_q$ is $CuBr_2$. In some embodiments, the reducing agent is an inorganic compound. In some embodiments, the reducing agent is selected from the group consisting of a sulfur compound of a low oxidation level, sodium hydrogen sulfite, an inorganic salt comprising a metal ion, a metal, hydrazine hydrate and derivatives of such compounds. In some embodiments, the reducing agent is a metal. In some embodiments, the reducing agent is Cu^0.

[0396] In some embodiments, the reducing agent is an organic compound. In some embodiments, the organic compound is selected from the group consisting of alkylthiols, mercaptoethanol, or carbonyl compounds that can be easily enolized, ascorbic acid, acetyl acetonate, camphosulfonic acid, hydroxy acetone, reducing sugars, monosaccharides, glucose, aldehydes, and derivatives of such organic compounds.

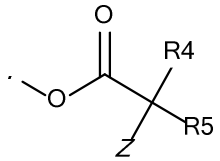
[0397] In some embodiments, the ligand is selected from the group consisting of 2,2'-bipyridine, 4,4'-Di-5-nonyl-2,2'-bipyridine, 4,4-dinonyl-2,2'-dipyridyl, 4,4',4''-tris(5-nonyl)-2,2':6',2''-terpyridine, N,N,N',N',N''-Pentamethyldiethylenetriamine, 1,1,4,7,10,10-Hexamethyltriethylenetetramine, Tris(2-dimethylaminoethyl)amine**,** N,N-bis(2-pyridylmethyl)octadecylamine, N,N,N',N'-tetra[(2-pyridal)methyl]ethylenediamine, tris[(2-pyridyl)methyl]amine**,** tris(2-aminoethyl)amine, tris(2-bis(3-butoxy-3-oxopropyl)aminoethyl)amine, tris(2-bis(3-(2-ethylhexoxy)-3-oxopropyl)aminoethyl)amine and Tris(2-bis(3-dodecoxy-3-oxopropyl)aminoethyl)amine. In some embodiments, the ligand is 2,2'-bipyridine.

[0398] In some embodiments the initiator has the structure:



Formula (22)

wherein R1 is a nucleophilic reactive group, R2 comprises a linker, and R3 comprises a polymer synthesis initiator moiety having the structure

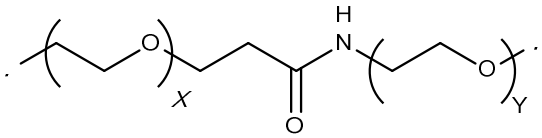


Formula (23)

wherein R4 and R5 and are the same or different and are selected from the group consisting of alkyl, substituted alkyl, alkylene, alkoxy, carboxyalkyl, haloalkyl, cycloalkyl, cyclic alkyl ether, alkenyl, alkenylene, alkynyl, alkynylene, cycloalkylene, heterocycloalkyl, heterocycloalkylene, aryl, arylene, arylene-oxy, heteroaryl, amino, amido or any combination thereof; Z is a halogen or CN; and s is an integer between 1 and 20.

[0399] In some embodiments, Z in Formula (23) is Br and R4 and R5 are each methyl. In some embodiments, R1 in Formula (22) is selected from the group consisting of NH_2-, OH-, and SH-.

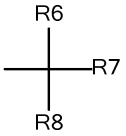
[0400] In some embodiments R2 in Formula (22) is alkyl, substituted alkyl, alkylene, alkoxy, carboxyalkyl, haloalkyl, cycloalkyl, cyclic alkyl ether, alkenyl, alkenylene, alkynyl, alkynylene, cycloalkylene, heterocycloalkyl, heterocycloalkylene, aryl, arylene, arylene-oxy, heteroaryl, amino, amido or any combination thereof. In some embodiments, R2 in Formula (22) is



Formula (24)

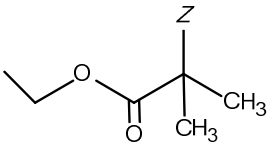
wherein X and Y are the same or different and are integers from 1-20. In some embodiments, X and Y are each 4.

[0401] In some embodiments, R3 in Formula (22) is

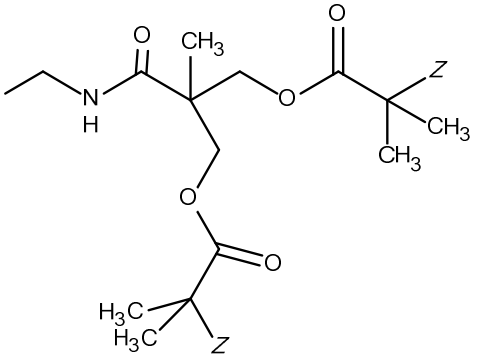


Formula (25)

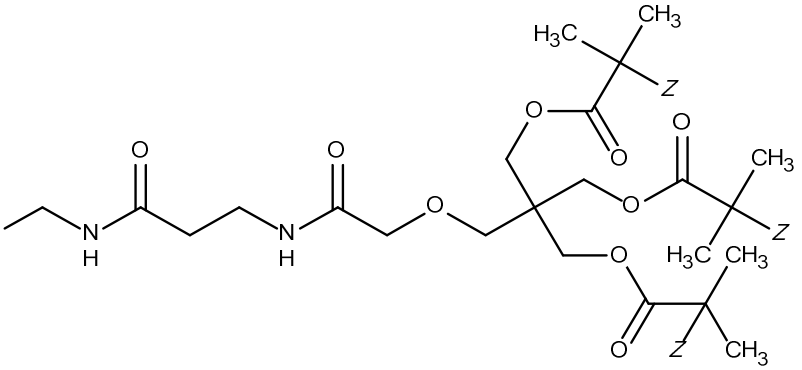
wherein R6, R7 and R8 are the same or different and are selected from the group consisting of



Formula (26),

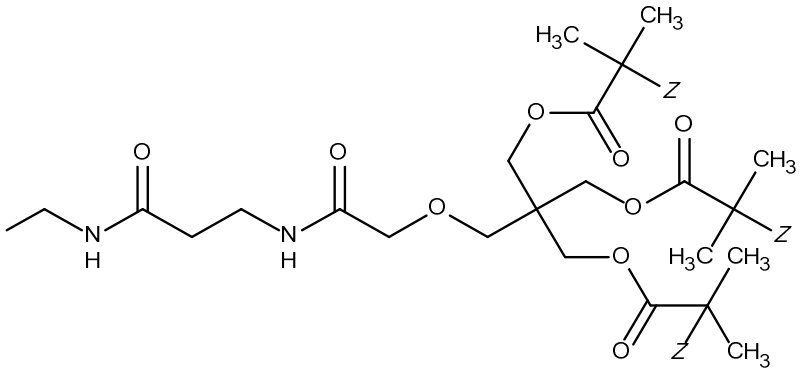


Formula (27), and



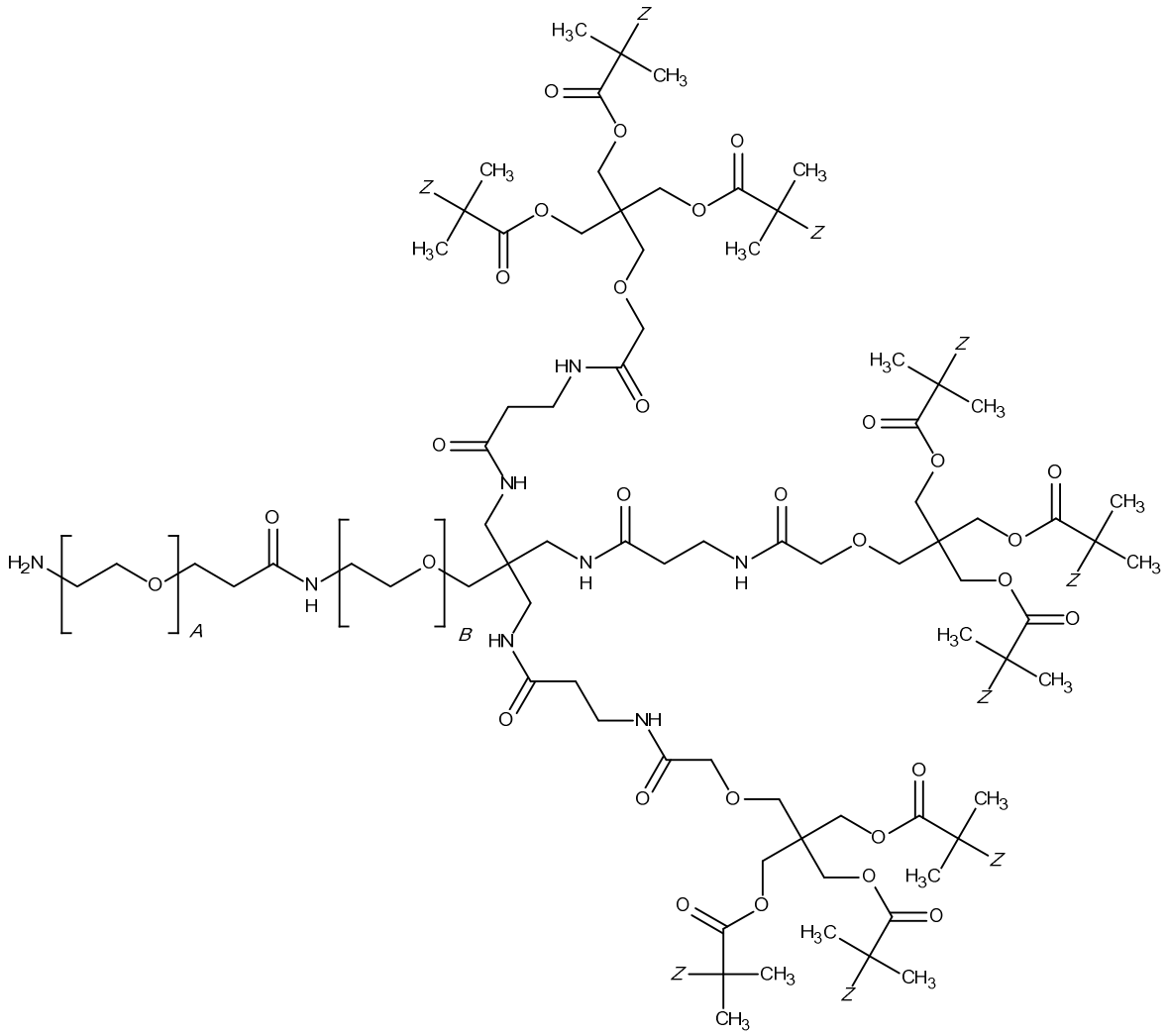
Formula (28)

wherein Z is NCS, F, Cl, Br or I. In some embodiments, Z in Formula (26), Formula (27) and/or Formula (28) is Br and R6, R7 and R8 in Formula (25) are each



Formula (29).

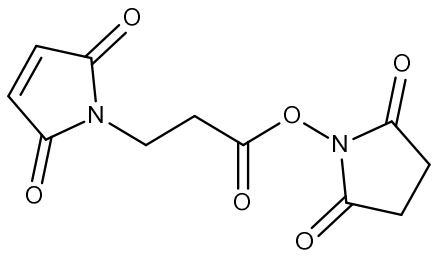
[0402] In some embodiments, the initiator has the structure:



Formula (30)

wherein A and B are the same or different and are integers from 2 to 12 and Z is any halide, for example Br. In some embodiments, A and B are each 4 in Formula (30).

[0403] In some embodiments, the method further has the step of reacting the polymer with a maleimide reagent to provide a polymer having a terminal maleimide. In some embodiments, the maleimide compound is



Formula (31).

[0404] A modification or mutation may also be made in a framework region or constant region to increase the half-life of an IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap). See, e.g., PCT Publication No. WO 00/09560. A mutation in a framework region or constant region can also be made to alter the immunogenicity of the antibody, to provide a site for covalent or non-covalent binding to another molecule, or to alter such properties as complement fixation, FcR binding and antibody-dependent cell-mediated cytotoxicity. In some embodiments, no more than one to five conservative amino acid substitutions are made within the framework region or constant region. In other embodiments, no more than one to three conservative amino acid substitutions are made within the framework region or constant region. According to the invention, a single antibody may have mutations in any one or more of the CDRs or framework regions of the variable domain or in the constant region.

[0405] Modifications also include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, 1997, Chem. Immunol. 65:111-128; Wright and Morrison, 1997, TibTECH 15:26-32). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al., 1996, Mol. Immunol. 32:1311-1318; Wittwe and Howard, 1990, Biochem. 29:4175-4180) and the intramolecular interaction between portions of the glycoprotein, which can affect the conformation and presented three-dimensional surface of the glycoprotein (Jefferis and Lund, supra; Wyss and Wagner, 1996, Current Opin. Biotech. 7:409-416). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, antibodies produced by CHO cells with tetracycline-regulated expression of β(1,4)-N-

acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana et al., 1999, Nature Biotech. 17:176-180).

[0406] **[]** Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine, asparagine-X-threonine, and asparagine-X-cysteine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0407] Addition of glycosylation sites to the antibody (and/or Ab-Trap) is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0408] The glycosylation pattern of antibodies (and/or Ab-Trap) may also be altered without altering the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g. antibodies, as potential therapeutics is rarely the native cell, variations in the glycosylation pattern of the antibodies can be expected (see, e.g. Hse et al., 1997, J. Biol. Chem. 272:9062-9070).

[0409] In addition to the choice of host cells, factors that affect glycosylation during recombinant production of antibodies (and/or Ab-Trap) include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U.S. Patent Nos. 5,047,335; 5,510,261 and 5,278,299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example, using endoglycosidase H (Endo H), N-glycosidase F, endoglycosidase F1, endoglycosidase F2, endoglycosidase F3. In addition,

the recombinant host cell can be genetically engineered to be defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

[0410] Some embodiments provided herein can be employed as a therapeutic for inflammatory retinal diseases and/or retinal vesicular diseases with a component of inflammation. In addition to angiogenesis, inflammation has been implicated in the pathogenesis of these retinal diseases. Anti-inflammatory therapies such as steroids have been effective in treating both uveitis (a spectrum of diseases with intraocular inflammation as a defining characteristic) and diabetic macular edema (DME). Similarly, genetically inherited polymorphisms in IL-6 have been associated with higher PDR incidence in patients with type 2 diabetes. Moreover, disease progression in AMD, DR and RVO have been reported to be associated with increased serum and/or ocular levels of IL-6. Additionally, chronic inflammatory cells have been seen on the surface of the Bruch's membrane in eyes with neovascular AMD. IL-6 has been implicated in resistance to anti-VEGF treatments in DME patients. This in part is believed to be an indirect result of IL-6 mediated upregulation of VEGF expression [reference] as well as more direct VEGF-independent angiogenic functions mediated by IL-6 signaling that occur in the presence of VEGF inhibitors. In some embodiments, any one or more of these conditions can be treated and/or prevented by one or more of the compositions provided herein.

[0411] FIG. 11C illustrates some embodiments of a construct of a bioconjugate, which is a Trap-Antibody Fusion (TAF) of VEGF trap (VEGFR1/2) and Anti-IL-6 antibody conjugated with phosphorylcholine-based biopolymer. In some embodiments, Anti-VEGF / anti-IL-6 bioconjugate can have a molecular weight of 1.0 MDa, with a clinical dose of 6.0 mg, in some embodiments. The equivalent molar dose of anti-VEGF portion in bioconjugate can be 6 times that of clinical Ranibizumab dose, while the ocular half-life could be 3-5 times of that of Ranibizumab. CH represents constant heavy, CL represents constant light, Fab represents fragment antigen-binding, Fc represents fragment crystallizable, VEGFR represents vascular endothelial growth factor receptor, VH represents variable heavy, VL represents variable light, and * are CDR regions. Equivalent values are showed as fold changes relative to Ranibizumab.

[0412] Various sequence arrangements of the Anti-IL-6 antibody / VEGF trap (VEGFR1/2) can be employed or provided in different arrangements. FIG. 21 illustrates some embodiments of possible anti-IL-6 heavy chain variable region sequences. The CDRs are underlined.

[0413] Based on the results with the VEGF trap variants and improved Anti-IL-6 paratopes, 216 molecules in two different configurations were designed and prepared that comprised combinations of sequences as displayed in FIGs. 19, 20, 21, 22 and 23. For the first configuration (VEGFR-Anti-IL-6) the VEGF trap, as shown by sequences 1A-1D of FIG. 19 is positioned at the beginning of the protein, followed by a double repeat of a Gly-Gly-Gly-Gly-Ser linker (GS), as shown by sequence 2A of FIG. 20, which connects the trap to the N-terminus of the anti-IL-6 heavy chain, as shown by sequences 3A-3I of FIG. 21. These constructs can be paired with light chains listed as sequences 4A-4C of FIG. 22. In some embodiments, the VEGF trap (from FIG. 19) can be combined with any of the anti-IL-6 light chains and/or heavy chaings shown in any of the figures or tables provided herein.

[0414] In some embodiments, any of the VEGF trap variants provided herein can be paired with any of: the heavy and light chain sequences of IL-6 antibodies provided herein, and can be further modified by any of the polymers provided herein. In some embodiments, any of the VEGF trap variants provided herein can be paired with any of: the heavy and light chain variable sequences of IL-6 antibodies provided herein, and can be further modified by any of the polymers provided herein. In some embodiments, any of the VEGF trap variants provided herein can be paired with any of: the heavy and light chain 3 CDRs (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3) of IL-6 antibodies provided herein, and can be further modified by any of the polymers provided herein. In some embodiments, the IL-6-VEGF trap fusion comprises the components in FIG. 27.

[0415] In some embodiments, the sequence of the anti-IL-6-VEGF trap (or dual inhibitor, fusion protein, or conjugate thereof) includes SEQ ID NO: 169 as the light chain, SEQ ID NO: 170 as the heavy chain fused to the VEGFR trap, via a linker. In some embodiments, the sequence of the anti-IL-6-VEGF trap (or dual inhibitor, or fusion protein or conjugate thereof) is at least 80% identical to a molecule comprising SEQ ID NO: 169 and 170, including, for example, at least 85, 90, 95, 96, 97, 98, 99, or greater identity to the combination of SEQ ID NO: 170 and 169. In some embodiments, the sequence of the anti-IL-6-VEGF trap (or conjugate thereof) is at least 80% identical to a molecule comprising SEQ ID NO: 169 and at least 80% identical to the molecule comprising SEQ ID NO: 170, including, for example, at least 85, 90, 95, 96, 97, 98, 99%, or greater identity to SEQ ID NO: 170 and at least 85, 90, 95, 96, 97, 98, 99%, or greater identity to 169. In some embodiments, the CDRs remain as depicted in any of the CDRs provided herein

for an anti-IL-6 antibody (including those in SEQ ID NO: 170 and 169), while the remainder of the sequences (heavy and light chain, heavy and light variable regions, and/or full fusion sequences (such as SEQ ID NO 169 and 170) are allowed to be vary such that the full sequence is at least 80, 85, 90, 95, 96, 97, 98, 99, or greater identity to the original sequence. In some embodiments, the CDRs can vary by 1, 2, or 3 conservative alterations, while the remainder of the sequences (heavy and light chain, heavy and light variable regions, and/or full fusion sequences (such as SEQ ID NO 169 and 170) are allowed to be vary such that the full sequence is at least 80, 85, 90, 95, 96, 97, 98, 99, or greater identity to the original sequence. In some embodiments, the trap sequence (that shown in gray in FIG. 27, or in FIG. 19) can retain at least 80, 85, 90, 95, 96, 97, 98, 99, or greater percent identity, and the CDRs can vary by 1, 2, or 3 conservative alterations, while the remainder of the sequences (heavy and light chain, heavy and light variable regions, and/or full fusion sequences (such as SEQ ID NO 169 and 170) are allowed to be vary such that the full sequence is at least 80, 85, 90, 95, 96, 97, 98, 99, or greater identity to the original sequence.

[0416] In some embodiments, the fusion protein (or dual inhibitor) or conjugate thereof comprises 1, 2, 3, 4, 5, or all 6 of the CDRs provided herein. In some embodiments, the fusion protein or conjugate thereof includes at least SEQ ID NOs: 49, 50, and 51 (or a variant with 1, 2, or 3 conservative substitutions). In some embodiments, the fusion protein (or dual inhibitor) or conjugate thereof includes at least SEQ ID NO:s 172, 173, and 174 (or a variant with 1, 2, or 3 conservative substitutions). In some embodiments, the fusion protein or conjugate thereof includes at least SEQ ID NO:s 49, 50, and 51 (or a variant with 1, 2, or 3 conservative substitutions) and at least SEQ ID NO:s 172, 173, and 174 (or a variant with 1, 2, or 3 conservative substitutions). In some embodiments, these 6 CDRs (or variants thereof) are contained within a human antibody framework region. In some embodiments, the CDRs are within the framework region of any of the antibodies provided herein, or variants thereof that are at least 80, 85, 90, 95, 96, 97, 98, 99, or greater percent identity to the heavy and/or light chain variable regions provided herein, including, without limitation, those in tables 1A for the heavy chain and 1B for the light chain. In some embodiments, the fusion protein (or dual inhibitor) or conjugate thereof includes at least SEQ ID NO:s 49, 50, and 51 (or a variant with 1, 2, or 3 conservative substitutions) and at least SEQ ID NO:s 172, 173, and 174 (or a variant with 1, 2, or 3 conservative substitutions) and these CDRs (including the variants) are substituted for the CDRs within one or more of the sequences in Table 1D, or within a variant within table 1D (where, disregarding the CDRs in Table 1D, the remaining

sequence is at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5 or greater percent identity to the heavy chain containing section and the light chain section in Table 1D). Thus, in some embodiments, these 6 CDRs (and the variants thereof) can be substituted not only for the CDRs of the sequences noted in Table 1, but also for variants thereof. In some embodiments, the CDRs provided in table 1E (including 1A1, 1E2, and 1E3) are excluded as options within the variants of possible CDRs. In some embodiment, the antibody (including fragments thereof) is one that is at least 80% identical to an antibody having the heavy and light chain variable regions depicted in FIG. 27, while maintaining the specific CDRs in FIG. 27 (so no variation within the CDR section in this embodiment).

[0417] In some embodiments, an isolated antagonistic IL-6 antibody is provided. It can comprise a heavy chain amino acid variable region that comprises a heavy chain that has a sequence of at least one of SEQ ID NOs: 7-13,19-27, 89, 90, 256-262; and a light chain amino acid variable region that comprises the light chain that has a sequences of at least one of SEQ ID NOs: 91-93, 28-30.

[0418] In some embodiments, an isolated antagonist IL-6 antibody is provided that comprises a heavy chain variable region (VH) comprising 3 complementarity determining regions: VH (CDR1), VH CDR2, and VH CDR3 having an amino acid sequence from the CDRs listed in SEQ ID NO: 256; and a light chain variable region (VL) comprising a VL CDR1, VL CDR2, and VL CDR3 having an amino acid sequence selected from the group of CDRs listed in SEQ ID NO: 91-93.

[0419] In some embodiments, an isolated antagonist antibody that binds to IL-6 is provide. The antibody comprises at least one of the following mutations based on EU numbering: L234A, L235A, and G237A.

[0420] In some embodiments, an isolated antagonistic antibody that binds to IL-6, the antibody comprising: a CDR_H1 that is a CDR_H1 in SEQ ID NO: 172; a CDR_H2 that is a CDR_H2 in SEQ ID NO: 173; a CDR_H3 that is a CDR_H3 in SEQ ID NO: 174: a CDR_L1 that is a CDR_L1 in SEQ ID NO: 199;a CDR_L2 that is a CDR_L2 in SEQ ID NO: 200; a CDR_L3 that is a CDR_L3 in SEQ ID NO: 201;at least one of the following mutations (EU numbering): L234A, L235A, and G237A; and at least one of the following mutations (EU numbering): Q347C or L443C.

[0421] In some embodiments, the VEGFR-Anti-IL-6 dual inhibitor comprises an anti-IL-6 heavy chain variable region sequences selected from SEQ ID NO: 7-13, 89, 90, and/or 256-262.

[0422] In some embodiments, the VEGFR-Anti-IL-6 dual inhibitor has a VEGF trap sequences selected from at least one of SEQ ID Nos: 145, 15, 16, or 17.

[0423] In some embodiments, the linker sequence is SEQ ID NO: 18.

[0424] In some embodiments, the heavy chain sequence for the Anti-IL-6 molecules is selected from at least one of SEQ ID NOs 19-27 or includes at least the sequence in one of SEQ ID NOs: 89, 90, 256-262.

[0425] In some embodiments, the light chain sequence for the anti-IL-6 molecule comprises at least 1, 2, or 3 light chain CDRs from at least one of SEQ ID NOs 76-84.

[0426] In some embodiments, the heavy chain sequence for the Anti-IL-6 molecule comprises at least 1, 2, or 3 heavy chain CDRs from at least one of SEQ ID NOs 49-75.

[0427] In some embodiments, the VEGFR-Anti-IL-6 dual inhibitor comprises a VEGFR-Fc sequence from at least one of SEQ ID NOs 85-88.

[0428] In some embodiments, the VEGFR-Anti-IL-6 dual inhibitor comprises one or more of the sequences in any one or more of SEQ ID Nos 7-13, 145, 15-17, 18-84.

[0429] In some embodiments, the VEGFR-Anti-IL-6 dual inhibitor comprises an IL-6 VH; an IL-6 VL; an IL-6 Fc; a VEGF Trap; and a linker. In some embodiments, the IL-6 VH comprises a sequence from an IL6 VH sequence in any one of SEQ ID NOs 19-27, 31-39, 89, 90, or 256-262. In some embodiments, the IL-6 VL comprises a sequence from an IL6 VL sequence in any one of SEQ ID Nos 28-30 or 91-93. In some embodiments, the Fc comprises a sequence from a Fc sequence in any one of SEQ ID NOs 40-48. In some embodiments, the VEGF Trap comprises a sequence from a VEGF trap sequence in any one of SEQ ID NOs: 145, 15, 16, or 17.

[0430] In some embodiments, a fusion protein is provided that comprises an IL-6 VH; an IL-6 VL; an IL-6 Fc; and a VEGF Trap, wherein the fusion protein alters HUVEC proliferation. In some embodiments, altering HUVEC proliferation is inhibiting VEGF/IL6 mediated proliferation.

[0431] In some embodiments, a fusion protein comprising a sequence that is at least 80% identical to SEQ ID NO: 263 and at least 80% identical to SEQ ID NO: 117 is provided. The fusion protein is further conjugated to a polymer. In some embodiments, the fusion protein is at

least 95% identical to SEQ ID NO: 263 and at least 95% identical to SEQ ID NO: 117. In some embodiments, the protein comprises at least a) SEQ ID NO: 172, 173, 174, 199, 200, and 201, or b) a substitutions of 1, 2, or 3 amino acids within SEQ ID NO: 172, 173, 174, 199, 200, and/or 201, wherein the substitution is a conservative substitution.

[0432] In some embodiments, any of the constructs provided herein can include one or more mutation at positions 94 and/or 95 of the VEGFR sequence. In some embodiments, the mutation(s) can be T94I and H95I. In some embodiments this reduces any cleavage of the VEGFR protein (as detailed in the examples below).

[0433] In some embodiments, (where Anti-IL-6-VEGFR is the orientation), the variable and constant domains of the heavy chain, illustrated by Heavy chain – Fab, sequences 5A-5I of FIG. 23, are connected to the VEGF trap, illustrated by sequences 1A-1D of FIG. 19, via the GS linker, illustrated by sequence 2A of FIG. 20, and then the Fc domain, illustrated by Heavy chain – Fc, sequences 5A-5I of FIG. 23, is fused to the C-terminal end of the VEGF trap. Thus, the VEGF trap is sandwiched between the antibody Fab and Fc regions. In some embodiments, these constructs can be paired with light chains listed on sequences 4A-4C of FIG. 22.

[0434] FIG. 19 illustrates some embodiments of VEGF trap sequences. Variation between the sequences is underlined and highlighted in bold. In some embodiments, any of the fusion constructs provided herein can include any of the highlighted and bolded variations provided in this, or any of the figures in the present specification.

[0435] FIG. 20 illustrates some embodiments of a double repeat Gly-Gly-Gly-Gly-Ser linker (GS) sequence. In some embodiments, more than one unit of sequence can be employed (for example, two, for a double repeat, three or more can be employed). In some embodiments, other linker sequences can be employed. In some embodiments, alternative linker sequences can be employed.

[0436] FIG. 21 illustrates heavy chain sequences for some embodiments of anti-IL-6 molecules. CDRs are underlined. In some embodiments, any of the sequences herein can be used in place of other heavy chain sequences that are part of fusion constructs in the present specification.

[0437] FIG. 22 illustrates some embodiments of light chain sequences for Anti-IL-6 molecules. CDRs are underlined. In some embodiments, any of the sequences herein can be used in place of other light chain sequences that are part of fusion constructs in the present specification.

[0438] FIG. 23 illustrates some embodiments of heavy chain (separated into Fab and Fc) sequences for Anti-IL-6 molecules. CDRs are underlined. In some embodiments, any of the sequences herein can be used in place of other heavy chain sequences that are part of fusion constructs in the present specification.

[0439] FIGs. 24A-24B illustrate the combinations of CDRs from FIGs. 21-23. FIG. 24A illustrates heavy chain CDR sequences as defined in FIGs. 21 and 23. FIG. 24B illustrates light chain CDR sequences as defined in FIG. 22. In some embodiments, rather than the entire heavy and/or light chain variable region being used in a construct, just the CDRs from one or more of the figures provided herein can be employed.

[0440] In some embodiments, the VEGFR-Fc trap fusion is part of an engineered IgG1 framework. In addition to the VEGFR-Anti-IL-6 and Anti-IL-6-VEGFR constructs, an anti-VEGF (VEGFR–Fc) molecule comprising the VEGF trap with cleavage resistant variants, as described above, and an engineered IgG1 Fc domain, as illustrated by sequences 6A-6D of FIG. 25, can be provided. The Fc domain for these molecules can contain L234A, L235A and G237A substitutions that minimize effector function, and L443C that allows site-specific conjugation with a half-life extending phosphorylcholine based biopolymer (residue positions follow EU numbering). FIG. 25 illustrates some embodiments of VEGFR-Fc sequence variants. Variation in the sequences is underlined and highlighted in bold. It is noted that these variations and/or combinations can provided a biotherapeutic that is further superior to and/or an alternative to other compounds currently used in therapy.

[0441] As shown in FIGs. 26A-26C, VEGFR-Fc and Eylea bind with similar affinity to VEGF. FIG. 26A illustrates the Biacore assays of VEGFR-Fc. FIG. 26B illustrates the Biacore assays of Eylea. FIG. 26C illustrates the experimental results (Ka, Kd, KD and Rmax) of VEGFR-Fc and Eylea.

[0442] In some embodiments, any one or more of the sequences for the specified amino acid sequence in any one or more of FIG. 18-23, and 25 can be swapped into the corresponding structure of any of the other embodiments provided herein or exchanged with any of the other sequences provided herein. For example, any of the sequences within FIG. 13C-13E, 19, or 25 can be used with any of the IL-6 heavy chains from FIG. 18, 21, or 23, with any of the linkers provided herein (e.g., FIG. 20), with any of the light chains (FIG. 22). In some embodiments, the construct is a VEGFR-Anti-IL-6 configuration and it includes a combination of one of sequences

1A-1D (FIG. 19), linked to the linker (e.g., FIG. 20, sequence 2A), linked to a heavy chain IL-6 sequence (e.g., FIG. 21, sequence 3A or 3B), linked to a light chain sequence (e.g., FIG. 22, sequence 4A). In some embodiments, any one of sequences 1A-1D (FIG. 19), can be combined with a linker (FIG. 20), and with a heavy chain anti-IL-6 sequence (FIG. 21, sequences 3A-3I), and with a light chain anti-IL-6 sequence (FIG. 22, sequence 4A-4C). In some embodiments, any of the other corresponding sequences for any particular unit of construct provided herein can be swapped into or in place of any one of these units.

[0443] In some embodiments, the dual inhibitor provides one or more synergistic result. In some embodiments, the dual inhibitor is synergistically better than each section administered as a monotherapy (e.g. an IL-6 therapy and a VEGF therapy combined). In some embodiments, the combined VEGFR-IL-6 construct provides a synergistic result, as shown in the proliferation assay results (e.g., where the dual inhibitor has a clear effect on HUVEC proliferation, an important component of angiogenesis, while neither Eylea nor Anti-IL-6 achieve any change). Thus, in some embodiments, a method for controlling HUVEC proliferation is provided, whereby one employs a VEGFR-Anti-IL-6 construct as provided herein.

[0444] In other embodiments, the fusion construct simply provides an additive benefit by achieving both VEGF trap results and anti-IL-6 results.

[0445] In some embodiments, any one or more of the components in any one of more of tables provided herein can be combined together such that a VEGFR-anti-IL-6 construct is produced or such that an anti-IL-6-VEGFR construct is produced. In some embodiments, the construct will also include an Fc region. In some embodiments, the Fc region can be a human Fc region.

[0446] In some embodiments, the CDRs alone (with or without any particular framework section) can be employed as the antibody section. Thus, any one or more (e.g., 3 or 6) of the CDRs within each chain or pair of chains (heavy and light) can be used from any of the tables or figures provided herein, in combination with any of the other components (VEGFR and/or linkers). CDRs and/or 1, 2, or 3 heavy chain CDRs) from any of the IL-6 constructs provided herein can be combined with any one or more of the VEGFR sequences provided herein. In some embodiments, any heavy and/or light chain variable region from any of the IL-6 constructs provided herein can be combined with any one or more of the VEGFR sequences provided herein. In some embodiments, any of the linkers can be provided within the final construct as well.

[0447] In some embodiments, any nucleic acid sequence encoding any one of more of the amino acid sequences provided herein can be provided as well (e.g., in isolation, within a vector, or in a cell, etc.)

[0448] In some embodiments, any one or more of the polymers for conjugation to form the bioconjugate can be combined with any of the VEGFR-IL6 constructs provided herein.

[0449] In some embodiments, the VEGFR-anti-IL-6 combination is sequence 1A-1D (FIG. 19) for the VEGFR, linked to a linker (2A in FIG. 20), linked to a heavy chain (3A or 3B in FIG. 21), associated with light chain 4A from FIG. 22.

[0450] In some embodiments, the VEGFR-anti-IL-6 combination is sequence 1A (FIG. 19) for the VEGFR, linked to a linker (2A in FIG. 20), linked to a heavy chain (3A in FIG. 21), associated with light chain 4A from FIG. 22. In some embodiments, the VEGFR-anti-IL-6 combination is sequence 1B (FIG. 19) for the VEGFR, linked to a linker (2A in FIG. 20), linked to a heavy chain (3A in FIG. 21), associated with light chain 4A from FIG. 22. In some embodiments, the VEGFR-anti-IL-6 combination is sequence 1C (FIG. 19) for the VEGFR, linked to a linker (2A in FIG. 20), linked to a heavy chain (3A in FIG. 21), associated with light chain 4A from FIG. 22. In some embodiments, the VEGFR-anti-IL-6 combination is sequence 1D (FIG. 19) for the VEGFR, linked to a linker (2A in FIG. 20), linked to a heavy chain (3A in FIG. 21), associated with light chain 4A from FIG. 22.

[0451] In some embodiments, the VEGFR-anti-IL-6 combination is sequence 1A (FIG. 19) for the VEGFR, linked to a linker (2A in FIG. 20), linked to a heavy chain (3B in FIG. 21), associated with light chain 4A from FIG. 22. In some embodiments, the VEGFR-anti-IL-6 combination is sequence 1B (FIG. 19) for the VEGFR, linked to a linker (2A in FIG. 20), linked to a heavy chain (3B in FIG. 21), associated with light chain 4A from FIG. 22. In some embodiments, the VEGFR-anti-IL-6 combination is sequence 1C (FIG. 19) for the VEGFR, linked to a linker (2A in FIG. 20), linked to a heavy chain (3B in FIG. 21), associated with light chain 4A from FIG. 22. In some embodiments, the VEGFR-anti-IL-6 combination is sequence 1D (FIG. 19) for the VEGFR, linked to a linker (2A in FIG. 20), linked to a heavy chain (3B in FIG. 21), associated with light chain 4A from FIG. 22.

[0452] In some embodiments, any of the IL-6 antibody sequences can be employed with any of the VEGFR sequences provided herein. In some embodiments, any 1, 2, 3, 4, 5, or 6

CDRS (1, 2, or 3 light chain and 1, 2, or 3 heavy chain) anti-IL-6 sequences can be employed with any of the VEGFR arrangements.

[0453] In some embodiments, any anti-IL-6 antibody light and/or heavy chain can be employed, with any one or more of the point mutations provided in any one of more of Tables 18.1-18.4. In some embodiments, the fusion construct employs an anti-IL-6 construct that has a point mutation at any one or more of the positions identified in any one or more of Tables 18.1-18.4. In some embodiments, the positions altered are those underlined and bolded in the relevant figures as showing changed residues. In some embodiments, the construct is one that includes changes in heavy chain include one or more of S35H, G66D, L100A, or L100S and the changes in light chain include one or more of M32L, M50D, N52S or M88Q. CDR positions follow their order of appearance in the variable domain when described (e.g. S35H, G66D…). Fc positions follow EU numbering when described (e.g. L234A, L235A….) In some embodiments, the construct (e.g., antibody to Il-6, and/or IL-6 VEGF Trap, and/or IL-6 VEGF Trap bioconjugate (to one or more of the biopolymers provided herein)), includes 1, 2, 3, 4, 5, 6, 7, or 8 of the following in the heavy and light chains: S35H, G66D, L100A, and/or L100S in the heavy and M32L, M50D, N52S and/or M88Q in the light chain. In some embodiments, these point mutations can be used in any one of the Il-antibodies or constructs containing Il-6 antibodies provided herein.

[0454] As will be appreciated by one of skill in the art, there are a variety of constructs provided herein. Generally, these constructs can be grouped into: antibodies to Il-6, and/or Anti-IL-6 VEGF Trap (fusions of the antibody and VEGF Trap), and/or IL-6 VEGF Trap bioconjugate (the fusions with one or more of the biopolymers provided herein), and/or VEGF Trap constructs, and/or VEGF Trap biopolymers. Thus, any of the separate components provided herein (and variants thereof) can be used separately or in combination with one another. In some embodiments, the constructs of any of these groupings (antibodies to Il-6, and/or anti-IL-6 VEGF Trap (fusions of the antibody and VEGF Trap), and/or IL-6 VEGF Trap bioconjugate (the fusions with one or more of the biopolymers provided herein), and/or VEGF Trap constructs, and/or VEGF Trap biopolymers) can be used for any of the methods or other compositions provided herein. For example, any of these options can be used for any of the methods of treatment that are described in regard to IL-6 VEGF Trap therapies, and/or IL-6 therapies, and/or IL-6 VEGF Trap bioconjugates. As will be appreciated, the characteristics and properties of each of the construct can be different for each of the treatments, in line with the properties of the components detailed

herein. Thus, the description provided herein with regard to, for example, cell lines, nucleic acids, therapies, and other embodiments, are not only specifically contemplated for anti-IL-6-VEGF Trap and/or bioconjugates thereof, but also for antibodies (and, for example, fragments thereof), antibody-bioconjugates, and VEGF Trap constructs separate from the anti-IL-VEGF Trap and/or bioconjugates. For example, in some embodiments, any of the point mutations (or combinations thereof) for IL-6 can be used in an antibody (or binding fragment thereof) for a treatment that is simply antibody based, instead of antibody and VEGF trap based arrangement. Thus, the point mutations for VEGF Trap and IL-6 antibodies is not limited to the context of fusion arrangements (although it does provide that), as it also describes such constructs (and uses thereof and method of making) separate from a fusion arrangement. Rather than repeat all such embodiments separately, it is noted that any such description for one of the arrangements (antibodies to Il-6, and/or Anti-IL-6 VEGF Trap (fusions of the antibody and VEGF Trap), and/or IL-6 VEGF Trap bioconjugate (the fusions with one or more of the biopolymers provided herein), and/or VEGF Trap constructs, and/or VEGF Trap biopolymers) applies and identifies the options for all of the other such arrangements (antibodies to Il-6, and/or Anti-IL-6 VEGF Trap (fusions of the antibody and VEGF Trap), and/or IL-6 VEGF Trap bioconjugate (the fusions with one or more of the biopolymers provided herein), and/or VEGF Trap constructs, and/or VEGF Trap biopolymers).

[0455] In some embodiments, any of the VEGF Trap constructs are contemplated for use as compositions, components, or therapies, including for example, those in FIGs. 13C-13E, 19, and 25 of the embodiments provided herein

[0456] In some embodiments, any of the anti-IL-6 antibody constructs are contemplated for use as compositions, components, or therapies, including for example, those in tables 1A and 1E and FIGs. 5, 18, 21, 22, 23, and 24 of the embodiments provided herein.

[0457] In some embodiments, the fusion protein comprises a mutation at position 94 in the VEGF Trap sequence, at position 95 in the VEGF Trap sequence, or at T94I and H95I in the VEGF Trap sequence.

[0458] In some embodiments, a VEGFR-Anti-IL-6 dual inhibitor is provided. The VEGFR-Anti-IL-6 dual inhibitor comprises a trap antibody fusion of Anti-IL 6 antibody and a VEGF (VEGFR1/2) trap, wherein the dual inhibitor includes at least one point mutation within a VEGFR sequence to reduce cleavage of the VEGFR sequence. In some embodiments, the VEGFR-Anti-IL-6 dual inhibitor has a molecular weight of 1.0 MDa.

[0459] In some embodiments, the VEGR-Anti-IL-6 dual inhibitor provides therapy for inflammatory retinal diseases.

[0460] In some embodiments, the VEGFR-Anti-IL-6 dual inhibitor comprises a constant heavy, constant light, a fragment antigen binding, a fragment crystallizable (Fc), vascular endothelial growth factor receptor (VEGFR), a variable heavy, a variable light and CDR regions.

[0461] In some embodiments, the Anti-IL-6 heavy chain variable region sequences can be selected from options VI, VII, VIII, IX, X, XI, or XII of FIG. 18

[0462] In some embodiments, the VEGF trap sequence can be selected from at least one of options 1A, 1B, 1C or 1D of FIG. 19.

[0463] In some embodiments for the VEGR-anti-IL-6 dual inhibitor, the heavy chain sequence for anti-IL-6 molecules can be selected from at least one of options 3A, 3B, 3C, 3D, 3E, 3F, 3G, 3H, or 3I of FIG. 21.

[0464] In some embodiments for the VEGFR-anti-IL-6 dual inhibitor the light chain sequence for Anti-IL-6 molecules comprises at least 1, 2, or 3 light chain CDRs from at least one of option 4A, 4B, or 4C of FIG. 24B.

[0465] In some embodiments for the VEGFR-anti-IL-6 dual inhibitor the heavy chain sequence for the anti-IL-6 molecule comprises at least 1, 2, or 3 heavy chain CDRs from at least one of option 5A, 5B, 5C, 5D, 5E, 5F, 5G, 5H, or 5I of FIG. 24A.

[0466] In some embodiments, the VEGFR-Anti-IL-6 dual inhibitor comprises a VEGFR-Fc sequence from at least one of option 6A, 6B, 6C or 6D of FIG. 25.

[0467] In some embodiments, the VEGFR-anti-IL-6 dual inhibitor comprises one or more of the sequences in FIGs. 18-25.

[0468] In some embodiments, the VEGFR-Anti-IL-6 dual inhibitor comprises an IL-6 VH, an IL-6 VL, an IL-6 Fc, a VEGF Trap, and a linker. In some embodiments, the IL-6 VH comprises a sequence from an IL6 VH sequence in FIGs. 21 or 23. In some embodiments, the IL-6 VL comprises a sequence from an IL6 VL sequence in FIGs. 22. In some embodiments, the Fc comprises a sequence from a Fc sequence in FIG 23. In some embodiments, the VEGF Trap comprises a sequence from a VEGF trap sequence.

[0469] In some embodiments, a protein construct is provided that comprises: at least 3 heavy chain CDRs; at least 3 light chain CDRs; a VEGF trap sequence; and a linker sequence, wherein each of the sequences is selected from a sequence within FIGs. 18-25.

[0470] In some embodiments, a fusion protein is provided that comprises: an IL-6 VH, an IL-6 VL, an IL-6 Fc, a VEGF Trap, and wherein the fusion protein alters HUVEC proliferation. In some embodiments, each sequence is selected from a sequence within FIGs. 18-25.

Polynucleotides, vectors, and host cells

[0471] The invention also provides polynucleotides encoding any of the antibodies (and/or IL-6 Ab-VEGF Trap), including antibody fragments and modified antibodies described herein. In another aspect, the invention provides a method of making any of the polynucleotides described herein. Polynucleotides can be made and expressed by procedures known in the art. Accordingly, the invention provides polynucleotides or compositions, including pharmaceutical compositions, comprising polynucleotides, encoding any of the IL-6 antagonists antibodies (and/or IL-6 Ab-VEGF Traps) provided herein.

[0472] Polynucleotides complementary to any such sequences are also encompassed by the present invention. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

[0473] Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes an antibody or a fragment thereof) or may comprise a variant of such a sequence. Polynucleotide variants contain one or more substitutions, additions, deletions and/or insertions such that the immunoreactivity of the encoded polypeptide is not diminished, relative to a native immunoreactive molecule. The effect on the immunoreactivity of the encoded polypeptide may generally be assessed as described herein. Variants preferably exhibit at least about 70% identity, more preferably, at least about 80% identity, yet more preferably, at least about 90% identity, and most preferably, at least about 95% identity to a polynucleotide sequence that encodes a native antibody or a fragment thereof.

[0474] Two polynucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for

maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, or 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

[0475] Optimal alignment of sequences for comparison may be conducted using the MegAlign® program in the Lasergene® suite of bioinformatics software (DNASTAR®, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O., 1978, A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., 1990, Unified Approach to Alignment and Phylogenes pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., 1989, CABIOS 5:151-153; Myers, E.W. and Muller W., 1988, CABIOS 4:11-17; Robinson, E.D., 1971, Comb. Theor. 11:105; Santou, N., Nes, M., 1987, Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R., 1973, Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J., 1983, Proc. Natl. Acad. Sci. USA 80:726-730.

[0476] Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

[0477] Variants may also, or alternatively, be substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing

under moderately stringent conditions to a naturally occurring DNA sequence encoding a native antibody (or a complementary sequence).

[0478] Suitable “moderately stringent conditions” include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1 % SDS.

[0479] As used herein, "highly stringent conditions" or "high stringency conditions" are those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt’s solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

[0480] It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

[0481] The polynucleotides of this invention can be obtained using chemical synthesis, recombinant methods, or PCR. Methods of chemical polynucleotide synthesis are well known in

the art and need not be described in detail herein. One of skill in the art can use the sequences provided herein and a commercial DNA synthesizer to produce a desired DNA sequence.

[0482] For preparing polynucleotides using recombinant methods, a polynucleotide comprising a desired sequence can be inserted into a suitable vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification, as further discussed herein. Polynucleotides may be inserted into host cells by any means known in the art. Cells are transformed by introducing an exogenous polynucleotide by direct uptake, endocytosis, transfection, F-mating or electroporation. Once introduced, the exogenous polynucleotide can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. The polynucleotide so amplified can be isolated from the host cell by methods well known within the art. See, e.g., Sambrook et al., 1989.

[0483] Alternatively, PCR allows reproduction of DNA sequences. PCR technology is well known in the art and is described in U.S. Patent Nos. 4,683,195, 4,800,159, 4,754,065 and 4,683,202, as well as PCR: The Polymerase Chain Reaction, Mullis et al. eds., Birkauswer Press, Boston, 1994.

[0484] RNA can be obtained by using the isolated DNA in an appropriate vector and inserting it into a suitable host cell. When the cell replicates and the DNA is transcribed into RNA, the RNA can then be isolated using methods well known to those of skill in the art, as set forth in Sambrook et al., 1989, supra, for example.

[0485] Suitable cloning vectors may be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors will generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, Bluescript (e.g., pBS SK+) and its derivatives, mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Strategene, and Invitrogen.

[0486] Expression vectors are further provided. Expression vectors generally are replicable polynucleotide constructs that contain a polynucleotide according to the invention. It is implied that an expression vector must be replicable in the host cells either as episomes or as an

integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (i.e., translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

[0487] The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (e.g., where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[0488] The invention also provides host cells comprising any of the polynucleotides described herein. Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of mammalian host cells include but not limited to COS, HeLa, and CHO cells. See also PCT Publication No. WO 87/04462. Suitable non-mammalian host cells include prokaryotes (such as *E. coli* or *B. subtillis*) and yeast (such as *S. cerevisae*, *S. pombe*; or *K. lactis*). Preferably, the host cells express the cDNAs at a level of about 5 fold higher, more preferably, 10 fold higher, even more preferably, 20 fold higher than that of the corresponding endogenous antibody or protein of interest, if present, in the host cells. Screening the host cells for a specific binding to IL-6 is effected by an immunoassay or FACS. A cell overexpressing the antibody or protein of interest can be identified.

[0489] An expression vector can be used to direct expression of an IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap). One skilled in the art is familiar with administration of expression vectors to obtain expression of an exogenous protein in vivo. See, e.g., U.S. Patent Nos. 6,436,908; 6,413,942; and 6,376,471. Administration of expression vectors includes local or systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration. In another embodiment, the expression vector is

administered directly to the sympathetic trunk or ganglion, or into a coronary artery, atrium, ventricle, or pericardium.

[0490] Targeted delivery of therapeutic compositions containing an expression vector, or subgenomic polynucleotides can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis et al., Trends Biotechnol., 1993, 11:202; Chiou et al., Gene Therapeutics: Methods And Applications Of Direct Gene Transfer, J.A. Wolff, ed., 1994; Wu et al., J. Biol. Chem., 1988, 263:621; Wu et al., J. Biol. Chem., 1994, 269:542; Zenke et al., Proc. Natl. Acad. Sci. USA, 1990, 87:3655; Wu et al., J. Biol. Chem., 1991, 266:338. Therapeutic compositions containing a polynucleotide are administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA can also be used during a gene therapy protocol. The therapeutic polynucleotides and polypeptides can be delivered using gene delivery vehicles. The gene delivery vehicle can be of viral or non-viral origin (see generally, Jolly, Cancer Gene Therapy, 1994, 1:51; Kimura, Human Gene Therapy, 1994, 5:845; Connelly, Human Gene Therapy, 1995, 1:185; and Kaplitt, Nature Genetics, 1994, 6:148). Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

[0491] Viral-based vectors for delivery of a desired polynucleotide and expression in a desired cell are well known in the art. Exemplary viral-based vehicles include, but are not limited to, recombinant retroviruses (see, e.g., PCT Publication Nos. WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; U.S. Patent Nos. 5, 219,740 and 4,777,127; GB Patent No. 2,200,651; and EP Patent No. 0 345 242), alphavirus-based vectors (e.g., Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532)), and adeno-associated virus (AAV) vectors (see, e.g., PCT Publication Nos. WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655). Administration of DNA linked to killed adenovirus as described in Curiel, Hum. Gene Ther., 1992, 3:147 can also be employed.

[0492] Non-viral delivery vehicles and methods can also be employed, including, but not limited to, polycationic condensed DNA linked or unlinked to killed adenovirus alone (see,

e.g., Curiel, Hum. Gene Ther., 1992, 3:147); ligand-linked DNA (see, e.g., Wu, J. Biol. Chem., 1989, 264:16985); eukaryotic cell delivery vehicles cells (see, e.g., U.S. Patent No. 5,814,482; PCT Publication Nos. WO 95/07994; WO 96/17072; WO 95/30763; and WO 97/42338) and nucleic charge neutralization or fusion with cell membranes. Naked DNA can also be employed. Exemplary naked DNA introduction methods are described in PCT Publication No. WO 90/11092 and U.S. Patent No. 5,580,859. Liposomes that can act as gene delivery vehicles are described in U.S. Patent No. 5,422,120; PCT Publication Nos. WO 95/13796; WO 94/23697; WO 91/14445; and EP 0524968. Additional approaches are described in Philip, Mol. Cell Biol., 1994, 14:2411, and in Woffendin, Proc. Natl. Acad. Sci., 1994, 91:1581.

Compositions

[0493] The invention also provides pharmaceutical compositions comprising an effective amount of an IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) conjugate described herein. Examples of such compositions, as well as how to formulate, are also described herein. In some embodiments, the composition comprises one or more IL-6 antagonist antibodies (and/or IL-6 Ab-VEGF Trap) and/or antibody (and/or Ab-Trap) conjugates. In other embodiments, the IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) recognizes human IL-6. In other embodiments, the IL-6 antagonist antibody is a human antibody. In other embodiments, the IL-6 antagonist antibody is a humanized antibody. In some embodiments, the IL-6 antagonist antibody comprises a constant region that does not trigger an unwanted or undesirable immune response, such as antibody-mediated lysis or ADCC. In other embodiments, the IL-6 antagonist (and/or IL-6 Ab-VEGF Trap) comprises one or more CDR(s) of the antibody (such as one, two, three, four, five, or, in some embodiments, all six CDRs).

[0494] It is understood that the compositions can comprise more than one IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) (e.g., a mixture of IL-6 antibodies that recognize different epitopes of IL-6). Other exemplary compositions comprise more than one IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) that recognize the same epitope(s), or different species of IL-6 antagonist antibodies (and/or IL-6 Ab-VEGF Trap) that bind to different epitopes of IL-6. In some embodiments, the compositions comprise a mixture of IL-6 antagonist antibodies (and/or IL-6 Ab-VEGF Trap) that recognize different variants of IL-6. In addition, in some embodiments, the composition can also, or instead, comprise more than one VEGF Trap. These alternative VEGF

Trap sections can include different sequences for the VEGF Trap section, as long as the sections at least have the same or similar functionality as noted herein.

[0495] The composition used in the present invention can further comprise pharmaceutically acceptable carriers, excipients, or stabilizers (Remington: The Science and practice of Pharmacy 20th Ed., 2000, Lippincott Williams and Wilkins, Ed. K. E. Hoover), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations, and may comprise buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, 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 Polysorbate, PLURONICS™ or polyethylene glycol (PEG). Pharmaceutically acceptable excipients are further described herein.

[0496] Pharmaceutical compositions adapted for parenteral administration include aqueous and non-aqueous sterile injection solution which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation substantially isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. Excipients which may be used for injectable solutions include water, alcohols, polyols, glycerin and vegetable oils, for example. The compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carried, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets. Pharmaceutical compositions can be substantially isotonic, implying an osmolality of about 250-350 mOsm/kg water.

[0497] The pharmaceutical compositions may contain preserving agents, solubilizing agents, stabilizing agents, wetting agents, emulsifiers, sweeteners, colorants, odorants, salts (substances of the present invention may themselves be provided in the form of a pharmaceutically acceptable salt), buffers, coating agents or antioxidants. They may also contain therapeutically active agents in addition to the substance of the present invention. The pharmaceutical compositions of the invention may be employed in combination with one or more pharmaceutically acceptable excipients. Such excipients may include, but are not limited to, saline, buffered saline (such as phosphate buffered saline), dextrose, liposomes, water, glycerol, ethanol and combinations thereof.

[0498] The IL-6 antagonist antibodies (and/or IL-6 Ab-VEGF Trap), IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) conjugates, and pharmaceutical compositions of the invention can be employed alone or in conjunction with other compounds, such as therapeutic compounds or molecules, e.g. anti-inflammatory drugs, analgesics or antibiotics. Such administration with other compounds may be simultaneous, separate or sequential. The components may be prepared in the form of a kit which may comprise instructions as appropriate.

Methods for preventing or treating ophthalmic disorders

[0499] In some embodiments, the antibodies (and/or IL-6 Ab-VEGF Trap) and the antibody (and/or IL-6 Ab-VEGF Trap) conjugates are useful in various applications including, but are not limited to, therapeutic treatment methods.

[0500] In some embodiments, a method for treating ocular disease, such as for example AMD is provided. In some embodiments, the method of treating AMD in a subject comprises administering to the subject in need thereof an effective amount of a composition (e.g., pharmaceutical composition) comprising any of the anti-IL-6 antibodies or IL6 Ab-VEGF Traps as described herein. As used herein, AMD includes dry AMD and wet AMD. In some embodiments, provided is a method of treating AMD in a subject, comprising administering to the subject in need thereof an effective amount of a composition comprising IL-6 antagonist antibodies or the IL-6 Ab-VEGF Traps or their conjugates as described herein. In some embodiments, the IL-6 antibody and/or IL-6 Ab VEGF Trap and/or conjugates thereof can be used to treat and/or prevent and/or reduce the risk of wet or neovascular macular degeneration, wet or neovascular age-related macular degeneration, dry age-related macular degeneration, venous, arterial or other blockage of the ocular and or retinal blood vessels with or without retinal edema, anterior and

posterior uveitis, uveitic macular edema, diabetic retinopathy, proliferative diabetic retinopathy, diabetic macular edema, non-proliferative diabetic macular edema, and intraocular tumors. In some embodiments, the antibody, or IL-6 Ab-VEGFTrap can be used for: (i) VEGF therapy-resistant patients, (ii) patients who lose efficacy to anti-VEGF agents due to the progression of additional underlying pathologies such as fibrosis, wound-healing, and atrophy and/or (iii) undertreatment due to the requirement for frequent intravitreal injections.

[0501] In some embodiments, a method for treating an ocular disease, such as a systemic disease, is provided. In some embodiments, systemic diseases that affect the eye such as Grave's disease or neuromyelitis optica, or systemic diseases that do not affect the eye such as multiple sclerosis, rheumatoid arthritis can be treated via the methods and compositions provided herein.

[0502] In some embodiments, the compositions provided herein can be used in combination with immune modulators such as PD-1/PDL-1 for oncology indications.

[0503] In some embodiments, the methods described herein further comprise a step of treating a subject with one or more additional form(s) of therapy. In some embodiments, the additional form of therapy is an additional AMD therapy including, but not limited to, VISUDYNE®, laser photocoagulation or intravitreal injection of, e.g., LUCENTIS®, MACUGEN®, EYLEA®, OZURDEX®, ILUVIEN®, TRIESENCE®, or TRIVARIS®.

[0504] With respect to all methods described herein, reference to IL-6 antagonist antibodies (and/or IL-6 Ab-VEGF Trap) also includes compositions comprising one or more additional agents. These compositions may further comprise suitable excipients, such as pharmaceutically acceptable excipients including buffers, which are well known in the art. The present invention can be used alone or in combination with other methods of treatment.

[0505] In some embodiments, a method for the treatment or prophylaxis of a disease in a patient in need thereof is provided. In some embodiments, the method comprises administering to the patient any of the isolated antagonist antibodies (and/or IL-6 Ab-VEGF Trap) disclosed herein. In some embodiments, the method comprises administering to the patient any of the conjugates disclosed herein. In some embodiments, the method comprises administering to the patient any of the compositions disclosed herein. In some embodiments, the method comprises identifying a patient having hyperactive IL-6 activity and administering to the patient any of the isolated antagonist antibodies disclosed herein. In some embodiments, the method comprises

identifying a patient having hyperactive IL-6 activity and administering to the patient any of the conjugates disclosed herein. In some embodiments, the method comprises identifying a patient having hyperactive IL-6 activity and administering to the patient any of the compositions disclosed herein. In some embodiments, the patient has elevated VEGF activity instead of, or in addition to, the elevated IL-6 activity.

[0506] In some embodiments, the disorder is selected from the group consisting of wet or neovascular macular degeneration, wet or neovascular age-related macular degeneration, dry age-related macular degeneration, venous, arterial or other blockage of the ocular and or retinal blood vessels with or without retinal edema, anterior and posterior uveitis, uveitic macular edema, , diabetic retinopathy, proliferative diabetic retinopathy, diabetic macular edema, non-proliferative diabetic macular edema, and intraocular tumors.

[0507] In some embodiments, the isolated antibody (and/or IL-6 Ab-VEGF Trap), conjugate (and/or IL-6 Ab-VEGF Trap-conjugate), composition or a combination thereof is administered no more frequently than once a month. In some embodiments, the isolated antibody, conjugate, composition or a combination thereof is administered no more frequently than once every two months. In some embodiments, the isolated antibody (and/or IL-6 Ab-VEGF Trap), conjugate (and/or IL-6 Ab-VEGF Trap)-conjugate, composition or a combination thereof is administered no more frequently than once every three months. In some embodiments, the isolated construct (e.g., Ab or Ab-Trap, with or without the polymer) is administered with a frequency between once a year and once every two months. In some embodiments, the treatment can be a single application of the antibody. In some embodiments, the treatment can be as many applications of the antibody as needed or desired for an outcome.

[0508] The IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) or conjugate thereof can be administered to a subject via any suitable route. It should be apparent to a person skilled in the art that the examples described herein are not intended to be limiting but to be illustrative of the techniques available. Accordingly, in some embodiments, the IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) is administered to a subject in accord with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, transdermal, subcutaneous, intra-articular, sublingually, intrasynovial, via insufflation, intrathecal, oral, inhalation or topical routes. Administration can be systemic, e.g., intravenous administration, or localized. Commercially

available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be directly nebulized and lyophilized powder can be nebulized after reconstitution. Alternatively, IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) can be aerosolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder.

[0509] In some embodiments, the IL-6 antagonist antibodies (and/or IL-6 Ab-VEGF Trap), IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) conjugates, and pharmaceutical compositions disclosed herein are used for prophylaxis or treatment of an ocular disease or condition. So used, the conjugates are typically formulated for and administered by ocular, intraocular, and/or intravitreal injection, and/or juxtascleral injection, and/or subtenon injection, and/or suprachoroidal injection and/or topical administration in the form of eye drops and/or ointment. Such IL-6 antagonist antibodies (and/or IL-6 Ab-VEGF Trap), IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) conjugates, and compositions can be delivered by a variety of methods, e.g. intravitreally as a device and/or a depot that allows for slow release of the compound into the vitreous, including those described in references such as Intraocular Drug Delivery, Jaffe, Jaffe, Ashton, and Pearson, editors, Taylor & Francis (March 2006). In one example, a device may be in the form of a minimum and/or a matrix and/or a passive diffusion system and/or encapsulated cells that release the compound for a prolonged period of time (Intraocular Drug Delivery, Jaffe, Jaffe, Ashton, and Pearson, editors, Taylor & Francis (March 2006). In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic or substantially isotonic.

[0510] Formulations for ocular, intraocular or intravitreal administration can be prepared by methods and using ingredients known in the art. Proper penetration into the eye is desirable for efficient treatment. Unlike diseases of the front of the eye, where drugs can be delivered topically, retinal diseases merit a more site-specific approach. Eye drops and ointments rarely penetrate the back of the eye, and the blood-ocular barrier hinders penetration of systemically administered drugs into ocular tissue. In some embodiments, the method of choice for drug delivery to treat retinal disease, such as AMD and CNV, is direct intravitreal injection. In some embodiments, intravitreal injections are repeated at intervals which depend on the patient's condition, and the properties and half-life of the drug delivered.

[0511] For administration to mammals, and particularly humans, it is expected that the dosage of the active agent is from 0.01 mg/kg body weight, to typically around 1 mg/kg, for systemic administrations. For ocular diseases that require local administration (for example, intravitreal, supracoroidal, peri-ocular etc), dosage is typically 0.1 mg/eye/dose to 10 mg/eye/dose or more. In some embodiments, the dosage is 100 ul/dose/eye. In some embodiments, a needle can be used to administer the dosage. The needle can be, for example, a 30 gauge ½ inch needle or a ½ inch needle that is 27G or 29G. The physician can determine the actual dosage most suitable for an individual which depends on factors including the age, weight, sex and response of the individual, the disease or disorder being treated and the age and condition of the individual being treated. The above dosages are exemplary of the average case. There can, of course, be instances where higher or lower dosages are merited.

[0512] This dosage may be repeated as often as appropriate (e.g., weekly, fortnightly, monthly, quarterly). If side effects develop the amount and/or frequency of the dosage can be reduced, in accordance with normal clinical practice. In one embodiment, the pharmaceutical composition may be administered once every one to thirty days.

[0513] The IL-6 antagonist antibodies (and/or IL-6 Ab-VEGF Trap) of the present invention may be employed in accordance with the instant invention by expression of such polypeptides in vivo in a patient, i.e., gene therapy. There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the patient's cells: *in vivo* and *ex vivo*. For in vivo delivery the nucleic acid is injected directly into the patient, usually at the sites where the therapeutic protein is required, i.e., where biological activity of the therapeutic protein is needed. For *ex vivo* treatment, the patient's cells are removed, the nucleic acid is introduced into these isolated cells, and the modified cells are administered to the patient either directly or, for example, encapsulated within porous membranes that are implanted into the patient (see, e.g., U.S. Pat. Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells in vitro, or transferred in vivo in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells in vitro include the use of liposomes, electroporation, microinjection, transduction, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. Transduction involves the association of a replication-defective, recombinant viral (preferably retroviral) particle with a cellular receptor, followed by introduction

of the nucleic acids contained by the particle into the cell. A commonly used vector for *ex vivo* delivery of the gene is a retrovirus.

[0514] The currently preferred *in vivo* nucleic acid transfer techniques include transfection with viral or non-viral vectors (such as adenovirus, lentivirus, Herpes simplex I virus, or adeno-associated virus (AAV)) and lipid-based systems (useful lipids for lipid-mediated transfer of the gene are, for example, DOTMA, DOPE, and DC-Chol; see, e.g., Tonkinison et al., Cancer Investigation, 14(1): 54-65 (1996)). The most preferred vectors for use in gene therapy are viruses, most preferably adenoviruses, AAV, lentiviruses, or retroviruses. A viral vector such as a retroviral vector includes at least one transcriptional promoter/enhancer or locus-defining element(s), or other elements that control gene expression by other means such as alternate splicing, nuclear RNA export, or post-translational modification of messenger. In addition, a viral vector such as a retroviral vector includes a nucleic acid molecule that, when transcribed in the presence of a gene encoding the therapeutic protein, is operably linked thereto and acts as a translation initiation sequence. Such vector constructs also include a packaging signal, long terminal repeats (LTRs) or portions thereof, and positive and negative strand primer binding sites appropriate to the virus used (if these are not already present in the viral vector). In addition, such vector typically includes a signal sequence for secretion of the PRO polypeptide from a host cell in which it is placed. Preferably the signal sequence for this purpose is a mammalian signal sequence, most preferably the native signal sequence for the therapeutic protein. Optionally, the vector construct may also include a signal that directs polyadenylation, as well as one or more restriction sites and a translation termination sequence. By way of example, such vectors will typically include a 5′ LTR, a tRNA binding site, a packaging signal, an origin of second-strand DNA synthesis, and a 3′ LTR or a portion thereof. Other vectors can be used that are non-viral, such as cationic lipids, polylysine, and dendrimers.

[0515] In some situations, it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell-surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins that bind to a cell-surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, e.g., capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins that undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated

endocytosis is described, for example, by Wu et al., J. Biol. Chem .,262: 4429-4432 (1987); and Wagner et al., Proc. Natl. Acad. Sci. USA, 87: 3410-3414(1990). For a review of the currently known gene marking and gene therapy protocols, see, Anderson et al., Science, 256: 808-813 (1992). See also WO 93/25673 and the references cited therein.

[0516] Suitable gene therapy and methods for making retroviral particles and structural proteins can be found in, e.g., U.S. Pat. No. 5,681,746.

[0517] In accordance some aspects, a method for treatment or prophylaxis of an ocular disease in a mammal is presented in which a nucleic acid molecule that encodes an IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) is administered.

[0518] In some embodiments, these IL6/VEGF dual inhibitor molecules can be used to treat ocular disorders such as the ophthalmic inflammatory disease scleritis, non-proliferative diabetic retinopathy, proliferative diabetic retinopathy (PDR), diabetic macular edema, prevention of diabetic macular edema, prevention of proliferative diabetic retinopathy, wet age-related macular degeneration, prevention of wet age-related macular degeneration, uveitis, and uveitic macular edema.

[0519] The VEGFR-AntiIL6 therapeutics could also be used to treat systemic diseases that affect the eye such as Grave's disease or neuromyelitis optica, or systemic diseases that do not affect the eye such as multiple sclerosis, rheumatoid arthritis.

[0520] These molecules could also be used to treat cytokine release syndrome following CAR-T or similar immune-oncology therapeutics. Additionally, anti-IL6 molecules abrogate the induction of IL-6 expression observed following treatment with anti-PD-1/PD-L1 molecules (Tsukamoto et al, 2018). Additionally, VEGF signaling blockade can also improve anti-PD-L1 treatment (Allen et al, 2017). Thus, these dual inhibitors could be used in combination with immune checkpoint inhibitors (such as PD-1/PDL-1 modulators) to synergistically treat cancer. VEGF inhibition would provide additional benefit on top of this. Another application of this could be used to cerebral edema in glioblastoma where anti-IL6 therapy may show additional benefits to anti-VEGF treatments. The biopolymer may also allow for increased tissue distribution, which would be beneficial for penetrating solid tumors.

[0521] In some embodiments, the conjugate (or the various proteins) is useful for prevention of any one or more of the disorders provided herein.

Formulations

[0522] Therapeutic formulations of the IL-6 antagonist antibodies (and/or IL-6 Ab-VEGF Trap) and IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) conjugates used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing, 2000), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and may comprise buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0523] Liposomes containing the IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) and/or IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) conjugate are prepared by methods known in the art, such as described in Epstein, et al., Proc. Natl. Acad. Sci. USA 82:3688 (1985); Hwang, et al., Proc. Natl Acad. Sci. USA 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

[0524] The active ingredients may also 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, The Science and Practice of Pharmacy 20th Ed. Mack Publishing (2000).

[0525] Sustained-release preparations 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. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

[0526] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes. Therapeutic IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) and/or antibody (and/or IL-6 Ab-VEGF Trap) conjugate compositions are generally placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle. In some embodiments, the antibody and/or antibody conjugate compositions are placed into a syringe to provide a prefilled syringe.

[0527] In some embodiments, the composition is a pyrogen-free composition which is substantially free of endotoxins and/or related pyrogenic substances. Endotoxins include toxins that are confined inside a microorganism and are released only when the microorganisms are broken down or die. Pyrogenic substances also include fever-inducing, thermostable substances from the outer membrane of bacteria and other microorganisms. Both of these substances can cause fever, hypotension and shock if administered to humans. Due to the potential harmful effects, even low amounts of endotoxins must be removed from intravenously administered pharmaceutical drug solutions. For systemic injection such as IV or IP, the Food & Drug Administration ("FDA") has set an upper limit of 5 endotoxin units (EU) per dose per kilogram body weight in a single one hour period for intravenous drug applications (The United States Pharmacopeial Convention, Pharmacopeial Forum 26 (1):223 (2000)). When therapeutic

proteins are administered in amounts of several hundred or thousand milligrams per kilogram body weight, as can be the case with proteins of interest (e.g., antibodies), even trace amounts of harmful and dangerous endotoxin must be removed. In some embodiments, the endotoxin and pyrogen levels in the composition are less than 10 EU/mg, or less than 5 EU/mg, or less than 1 EU/mg, or less than 0.1 EU/mg, or less than 0.01 EU/mg, or less than 0.001 EU/mg. In some embodiments, the compositions or methods provided herein allow for 0.1EU/eye/injection. In some embodiments, the compositions or methods provided herein allow for 0.05EU/eye/injection. In some embodiments, the compositions or methods provided herein allow for 0.02EU/eye/injection. In some embodiments, the compositions or methods provided herein allow for 0.01EU/eye/injection.

[0528] The compositions according to the present invention may be in unit dosage forms such as tablets, pills, capsules, powders, granules, solutions or suspensions, or suppositories, for oral, parenteral or rectal administration, or administration by inhalation or insufflation.

[0529] For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical carrier, e.g. conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other pharmaceutical diluents, e.g. water, to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention, or a non-toxic pharmaceutically acceptable salt thereof. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type described above containing from about 0.1 to about 500 mg of the active ingredient of the present invention. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer that serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

[0530] Suitable surface-active agents include, in particular, non-ionic agents, such as Polysorbate or polyoxyethylenesorbitans (e.g. Tween™ 20, 40, 60, 80 or 85) and other sorbitans (e.g. Span™ 20, 40, 60, 80 or 85). Compositions with a surface-active agent will conveniently comprise between 0.01%, and 5% surface-active agent, and can be between 0.01 and 0.02% or 0.1 and 2.5% (polysorbate 20 or 80). It will be appreciated that other ingredients may be added, for example mannitol or other pharmaceutically acceptable vehicles, if necessary.

[0531] Suitable emulsions may be prepared using commercially available fat emulsions, such as INTRALIPID™, LIPOSYN™, INFONUTROL™, LIPOFUNDIN™ and LIPIPHYSAN™. The active ingredient may be either dissolved in a pre-mixed emulsion composition or alternatively it may be dissolved in an oil (e.g. soybean oil, safflower oil, cottonseed oil, sesame oil, corn oil or almond oil) and an emulsion formed upon mixing with a phospholipid (e.g. egg phospholipids, soybean phospholipids or soybean lecithin) and water. It will be appreciated that other ingredients may be added, for example glycerol or glucose, to adjust the tonicity of the emulsion. Suitable emulsions will typically contain up to 20% oil, for example, between 5 and 20%. The fat emulsion can comprise fat droplets between 0.1 and 1.0 μm, particularly 0.1 and 0.5 μm, and have a pH in the range of 5.5 to 8.0.

[0532] The emulsion compositions can be those prepared by mixing an IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) with Intralipid™ or the components thereof (soybean oil, egg phospholipids, glycerol and water).

[0533] Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as set out above. In some embodiments, the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably sterile pharmaceutically acceptable solvents may be nebulised by use of gases. Nebulised solutions may be breathed directly from the nebulising device or the nebulising device may be attached to a face mask, tent or intermittent positive pressure breathing machine. Solution, suspension or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

Kits

[0534] The invention also provides kits comprising any or all of the antibodies described herein. Kits of the invention include one or more containers comprising an IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) or conjugate described herein and instructions for use in accordance with any of the methods of the invention described herein. Generally, these instructions comprise a description of administration of the IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) or conjugate for the above described therapeutic treatments. In some embodiments, kits are provided for producing a single-dose administration unit. In certain embodiments, the kit can contain both a first container having a dried protein and a second container having an aqueous formulation. In certain embodiments, kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes) are included.

[0535] In some embodiments, the antibody is a human antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a monoclonal antibody. The instructions relating to the use of an IL-6 antagonist antibody (and/or IL-6 Ab-VEGF Trap) or conjugate generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

[0536] The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as prefilled syringe, an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit 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). The container may also 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 IL-6 antagonist antibody or conjugate. The container may further comprise a second pharmaceutically active agent.

[0537] Kits may optionally provide additional components such as buffers and interpretive information. In some embodiments, the kits can include an additional syringe and needle used for back fill of the dosing syringe. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container.

[0538] The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

EXAMPLES

[0539] Despite the therapeutic success of anti-vascular endothelial growth factor (VEGF) biologic drugs for the treatment of neovascular retinal diseases, unmet need still exists due to (i) VEGF therapy-resistant patients, (ii) patients who lose efficacy to anti-VEGF agents due to the progression of additional underlying pathologies such as fibrosis, wound-healing, and atrophy (iii) undertreatment due to the requirement for frequent intravitreal injections. Below are results regarding novel bi-specific biologic agents designed to inhibit both VEGF and IL-6.

[0540] **Methods** Dual inhibitors were designed and characterized for their binding affinities to their respective targets by surface plasmon resonance (SPR). Functional aspects of the molecules were tested by (1) ELISA assays targeting the competitive blocking of each target to its respective receptor (2) VEGF and/or IL-6 stimulated primary human retinal microvascular endothelial cell (HRMVEC) proliferation assays can be performed, and (3) three-dimensional primary cell co-culture assays to assess the inhibition of angiogenic sprouting can be performed.

[0541] IL-6 Ab-VEGF Trap constructs bound with pM affinity to VEGF and IL-6. Binding kinetics of each target were independent of the presence of the other, indicating that both targets can bind simultaneously without interference. Binding of these molecules to each of their targets inhibits interactions with their respective cognate receptors.

[0542] When combined within a biopolymer conjugate platform, these dual inhibitor molecules provide a further therapeutic agent for the next generation of treatment and prevention of retinal diseases .

Example 1 - Antibody Generation and Screening

Methods/results:
Anti-IL-6

[0543] The present examples outline the construction of an anti-IL-6 and anti-VEGF dual inhibitor molecule containing an IL-6 antibody in an engineered human IgG1 framework and IgG1 Fc domain fused to an anti-VEGF trap. The Fc domain for these molecules contains L234A, L235A and G237A substitutions that minimize effector function, and L443C that allows site specific conjugation with a half-life extending phosphorylcholine based biopolymer (residue positions follow EU numbering).

[0544] An anti-IL-6 paratope in the engineered human IgG1 framework was affinity maturated using a combination of library scanning mutagenesis in *E. coli* followed by site-directed mutagenesis in a mammalian system. The construction of a dual expression plasmid system containing a heavy and light chain Fab with anti-IL-6 CDR sequences was achieved in *E. coli*. FIG. 3 depicts a flow chart of the process, which resulted in the antibody heavy and light chain variable regions in FIG. 5.

[0545] Fab libraries were constructed containing random single point mutations for all 71 CDR positions, excluding substitutions for Cys, Met and Asn due to the chemical liabilities associated with these amino acids. Small-scale expression in 96 well plates format was utilized for screening the Fab libraries. *E. coli* colonies (TG1 cells, Zymo Research) containing randomized single point mutations were picked into 96 well plates (1.5 mL) and grown overnight at 30 °C in LB Amp+2% glucose. A separate 96 well plate was created on the following day by removing 100 µL of culture and adding 100 µL of 30% glycerol, then stored at -80 °C. The remaining culture was spun down for 20 min and later resuspended in 1.5 mL of LB Amp containing 1 mM IPTG for induction of protein expression at 30 °C. After 5 hours, cells were spun down, resuspended in 500 µL of HBS-EP+ buffer and lysed by one freeze/thaw cycle.

Lysates were centrifuged for 30 minutes and vacuum filtered (200 µL) into round bottom 96 well plates for Biacore analysis.

[0546] Affinities of anti-IL-6 Fabs were determined using a Biacore T200 (GE Healthcare) at 25 °C. CM5 chips were activated with EDC and NHS, recombinant human IL-6 (R&D Systems) was diluted into 10 mM sodium acetate pH 5 to a concentration of 0.1 mg/mL and injected over the activated chip. Three ranges of antigen density (100-150 RU, 300-400 RU, 400-600 RU) were obtained by using variable flow times for each chip channel, later blocked with ethanolamine. Supernatants of the 96-well filtered culture lysates were injected at 50 µL/min for 120 seconds. Fitting of dissociation curves (300 seconds) to single exponential decay curve was used for the determination of dissociation rates (k_{off}). Chip surfaces were regenerated with 10 mM Glycine pH 1.7 solution injected at 50 µL/min during 60 seconds after every cycle of sample binding and dissociation. Clones that showed similar or improved k_{off} values in comparison to the wild-type were submitted for DNA sequencing for mutation identification.

[0547] From the total of 71 CDR residue positions, screening data showed 15 residues were critical for IL-6 binding and could not be replaced by any other amino acid. Substitutions in tolerable positions resulted in similar k_{off} values to the parent molecule (Table 1.1). Most of these single replacements were later introduced into the genes of the full heavy and light chains cloned in mammalian expression vectors, which were co-transfected into 3 ml cultures of Expi293 cells for protein production. On the fifth day, these cultures were centrifuged and the supernatants containing secreted antibodies were harvested and diluted in HBS-EP+ running buffer (1:20 ratio). Point mutants are in tables 1.1-1.6

TABLE 1.1

HEAVY CHAIN CDR1

	G26	F27	T28	F29	S30	P31	F32	A33	M34	S35
F						crit.		crit.		
L						crit.		crit.		
I						crit.		crit.		
V		4.05E-03				crit.	5.41E-03	crit.		
S	3.75E-03	3.93E-03				crit.		crit.		
P		4.18E-03				crit.		crit.		
T						crit.		crit.		
A			3.96E-03			crit.		crit.		
Y			3.90E-03	5.11E-03	3.52E-03	crit.		crit.		
H				5.24E-03		crit.		crit.		3.92E-03
Q						crit.		crit.		3.54E-03
K			4.08E-03			crit.		crit.		
D						crit.		crit.		
E			4.28E-03			crit.		crit.		
W					3.69E-03	crit.		crit.	3.97E-03	
R	3.81E-03	3.94E-03				crit.		crit.		
G		4.01E-03			3.77E-03	crit.	5.25E-03	crit.		

[0548] Table displays koff values (s-1) for every tolerable substitution within define CDR. In overall mutants present similar koff to parental molecule (4.94E-03 ±7.67E-04). Residues critical for IL-6 binding and did not tolerate replacements are indicated as 'crit'.

TABLE 1.2

HEAVY CHAIN CDR2

	V48	A49	K50	I51	S52	P53	G54	G55	S56	W57	T58	Y59	Y60	S61	D62	T63	V64	T65	G66
F			crit.		crit.	crit.	crit.	crit.		6.18E-03		crit.	crit.				6.04E-03		
L			crit.		crit.	crit.	crit.	crit.				crit.	crit.					5.92E-03	
I	6.17E-03		crit.		crit.	crit.	crit.	crit.				crit.	crit.					6.16E-03	
V			crit.		crit.	crit.	crit.	crit.			5.62E-03	crit.	crit.						
S		5.42E-03	crit.		crit.	crit.	crit.	crit.				crit.	crit.						
P			crit.		crit.	crit.	crit.	crit.				crit.	crit.						
T		5.71E-03	crit.		crit.	crit.	crit.	crit.				crit.	crit.						
A			crit.		crit.	crit.	crit.	crit.				crit.	crit.	6.04E-03			6.43E-03	6.15E-03	
Y			crit.		crit.	crit.	crit.	crit.				crit.	crit.					5.74E-03	
H			crit.		crit.	crit.	crit.	crit.	4.94E-03			crit.	crit.						
Q	5.40E-03		crit.		crit.	crit.	crit.	crit.				crit.	crit.		4.34E-03	5.70E-03			
K			crit.		crit.	crit.	crit.	crit.			4.92E-03	crit.	crit.	5.69E-03		6.11E-03			
D			crit.		crit.	crit.	crit.	crit.				crit.	crit.			6.03E-03		6.14E-03	6.07E-03
E			crit.		crit.	crit.	crit.	crit.				crit.	crit.			5.53E-03			5.82E-03
W			crit.		crit.	crit.	crit.	crit.				crit.	crit.						
R			crit.	3.15E-03	crit.	crit.	crit.	crit.	5.09E-03		4.25E-03	crit.	crit.	5.85E-03					
G			crit.		crit.	crit.	crit.	crit.				crit.	crit.			5.59E-03		5.70E-03	

[0549] Table displays koff values (s-1) for every tolerable substitution within define CDR. In overall mutants present similar koff to parental molecule (4.94E-03 ±7.67E-04). Residues critical for IL-6 binding and did not tolerate replacements are indicated as 'crit'.

TABLE 1.3

HEAVY CHAIN CDR3

	A97	R98	Q99	L100	W101	G102	Y103	Y104	A105	L106	D107	I108
F					crit.					6.01E-03		3.40E-03
L					crit.							
I					crit.							
V	4.12E-03				crit.			0.00381				
S					crit.		0.00502					3.52E-03
P					crit.							
T					crit.							
A			3.88E-03		crit.							
Y					crit.							
H			4.41E-03		crit.				0.00378			
Q				2.45E-03	crit.				0.00401			
K				2.87E-03	crit.							
D					crit.	0.00389	0.00503					
E					crit.		0.00512				4.38E-03	3.08E-03
W					crit.							
R					crit.							
G		0.0048			crit.							

[0550] Table displays koff values (s-1) for every tolerable substitution within define CDR. In overall mutants present similar koff to parental molecule (4.94E-03 ±7.67E-04). Residues critical for IL-6 binding and did not tolerate replacements are indicated as 'crit'.

TABLE 1.4

LIGHT CHAIN CDR1

	S24	A25	S26	I27	S28	V29	S30	Y31	M32	Y33
F				5.94E-03				5.10E-03		6.18E-03
L				5.93E-03		5.43E-03			4.92E-03	
I	4.12E-03									
V	4.16E-03								4.55E-03	
S		4.80E-03								
P	3.97E-03									
T		4.79E-03								
A							6.54E-03			
Y										
H			4.27E-03							
Q										
K	4.16E-03		4.41E-03							
D										
E					4.51E-03					
W				5.69E-03	3.98E-03					
R										
G			3.94E-03				6.77E-03	5.11E-03		

[0551] Table displays koff values (s-1) for every tolerable substitution within define CDR. In overall mutants present similar koff to parental molecule (4.94E-03 ±7.67E-04). Residues critical for IL-6 binding and did not tolerate replacements are indicated as 'crit'.

TABLE 1.5

LIGHT CHAIN CDR2

	L45	L46	I47	Y48	D49	M50	S51	N52	L53	A54	S55
F	crit.			5.37E-03							
L	crit.									4.89E-03	5.07E-03
I	crit.										5.05E-03
V	crit.	5.03E-03	4.93E-03								5.16E-03
S	crit.							5.55E-03			
P	crit.										
T	crit.										5.20E-03
A	crit.										
Y	crit.									5.25E-03	5.08E-03
H	crit.								4.98E-03		
Q	crit.										
K	crit.						4.61E-03				
D	crit.					4.58E-03	4.66E-03				
E	crit.				6.19E-03	4.76E-03					
W	crit.			5.63E-03					5.01E-03		5.10E-03
R	crit.						4.67E-03		5.00E-03		5.26E-03
G	crit.									4.98E-03	

[0552] Table displays koff values (s-1) for every tolerable substitution within define CDR. In overall mutants present similar koff to parental molecule (4.94E-03 ±7.67E-04). Residues critical for IL-6 binding and did not tolerate replacements are indicated as 'crit'.

TABLE 1.6

LIGHT CHAIN CDR3

	M88	Q89	W90	S91	G92	Y93	P94	Y95	T96
F			crit.		crit.	crit.	crit.		
L			crit.		crit.	crit.	crit.		4.78E-03
I			crit.		crit.	crit.	crit.	6.08E-03	4.23E-03
V	4.95E-03		crit.		crit.	crit.	crit.		5.04E-03
S		6.01E-03	crit.		crit.	crit.	crit.		
P			crit.		crit.	crit.	crit.		
T			crit.		crit.	crit.	crit.		
A	4.79E-03	5.85E-03	crit.	5.59E-03	crit.	crit.	crit.		
Y			crit.		crit.	crit.	crit.		
H			crit.		crit.	crit.	crit.		
Q			crit.		crit.	crit.	crit.		
K			crit.	5.49E-03	crit.	crit.	crit.		
D			crit.		crit.	crit.	crit.		
E			crit.		crit.	crit.	crit.		
W			crit.		crit.	crit.	crit.		
R			crit.		crit.	crit.	crit.		
G			crit.		crit.	crit.	crit.	4.62E-03	

[0553] Table displays koff values (s-1) for every tolerable substitution within define CDR. In overall mutants present similar koff to parental molecule (4.94E-03 ±7.67E-04). Residues critical for IL-6 binding and did not tolerate replacements are indicated as 'crit'.

[0554] Binding kinetics of these single mutant molecules to IL-6 were measured at 25 °C using a Biacore T200. Antibodies were captured on a Protein A chip (GE) by injecting 1:20 dilution of cell supernatant at 10 µL/min for 60 seconds. Five concentrations (0.56, 1.67, 5, 15 and 45 nM) of IL-6 were flowed over captured antibodies for 60 seconds at a flow rate of 30 µL/min and dissociated for 180 seconds in a single cycle kinetics Biacore assay format. The sensor chip was regenerated by a 60 seconds injection of 10 mM Glycine pH 1.7 at flow rate of 50 µL/min. A similar assay setup was used to monitor interactions between purified antibody

samples and IL-6 at 37 °C, when applicable. For this setup, 1 µg/mL of purified antibody was injected over a Protein A chip at 10 µL/min for 25-28 seconds. Data analysis for supernatant and purified samples were performed using BIAevaluation software (GE). All sensorgrams were double reference subtracted and fit using a 1:1 Langmuir binding model.

[0555] From all single point mutations tested (Table 1.7), the G66D mutation at the heavy chain CDR2 (sequential numbering of the antibody variable domain) resulted in a marked affinity boost (~2 fold decrease in K_D relative to wild-type: 6.95×10^{-11} M vs 1.58×10^{-10} M respectively). G66D was retained, and the remaining heavy chain and light chain CDR residues that could have manufacturing related chemical liabilities (Cys, Met, Asn) were mutagenized. Based on this premise, two heavy chain variants were generated including triple mutations (Table 1.9): I) M34I/G66D/I108V, II) M34W/G66D/I108V and three light chain variants including triple and quadruple mutations (Table 1.10): III) M32L/M50D/N52S/M88Q, IV) M32L/M50A/N52S/M88Q, V) M32L/M50D/M88Q (residue positions follow sequential numbering of the antibody variable heavy chain). We evaluated the IL-6 binding to all the possible combinations among these variants using the same Biacore assay setup described for the single point mutations. Our findings demonstrated these mutations countered the effect of the single point G66D mutation while keeping the affinity to IL-6 closer to the parent CDR sequence (Table 1.8). Based on these results, these modified CDRs were incorporated into the design of the anti-IL-6 and IL-6 Ab-VEGF Trap.

TABLE 1.7

			Sup / 25 °C							Purified / 37 °C					
CDR	**Heavy chain - mutation**	**Light chain - mutation**	**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU)**	**Chi² (RU²)**		**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU)**	**Chi² (RU²)**
	WT	WT	981.4	3.15E+05	1.26E-04	3.99E-10	223.2	0.108		138.1	8.80E+05	1.39E-04	1.58E-10	34.6	0.397
Heavy	G26S	WT	437.8	5.51E+05	1.15E-04	2.09E-10	96.7	0.0358		172.4	8.42E+05	3.90E-04	4.63E-10	39.8	0.0688

			Sup / 25 °C							Purified / 37 °C					
CDR	Heavy chain - mutation	Light chain - mutation	Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)		Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
	G26R	WT	638.4	4.02E+05	1.21E-04	3.02E-10	141.5	0.0304		183.3	7.02E+05	3.26E-04	4.65E-10	42.8	0.185
	F27G	WT	764	8.56E+05	2.06E-04	2.40E-10	129.3	0.19							
	F27P	WT	734.7	5.70E+05	1.96E-04	3.44E-10	125.9	0.0913							
	F27R	WT	809.6	5.03E+05	2.09E-04	4.15E-10	137.7	0.0638							
	F27S	WT	573.2	7.31E+05	2.03E-04	2.78E-10	93.5	0.12							
	F27V	WT	699.4	4.70E+05	2.08E-04	4.42E-10	114	0.0548							
	T28Y	WT	797.6	3.32E+05	1.37E-04	4.12E-10	165.6	0.229							
	T28K	WT	773.6	3.86E+05	1.43E-04	3.70E-10	171	0.157							
	T28A	WT	705.1	3.93E+05	1.39E-04	3.54E-10	157.5	0.0635							

			Sup / 25 °C							Purified / 37 °C					
CDR	**Heavy chain - mutation**	**Light chain - mutation**	**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU)**	**Chi² (RU²)**		**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU)**	**Chi² (RU²)**
	T28E	WT	832.8	4.69E+05	1.63E-04	3.47E-10	192.9	0.112							
	F29H	WT	653.3	4.95E+05	1.35E-04	2.73E-10	148.1	0.0271		156.2	8.50E+05	3.97E-04	4.66E-10	37.2	0.0579
	F29Y	WT	623.6	4.99E+05	1.33E-04	2.66E-10	139.3	0.0423		174.1	8.42E+05	3.74E-04	4.44E-10	40.2	0.078
	S30W	WT	619.7	4.17E+05	1.57E-04	3.76E-10	127.9	0.145							
	S30Y	WT	545.2	3.65E+05	1.22E-04	3.34E-10	110.9	0.0853							
	S30G	WT	686.2	3.98E+05	1.32E-04	3.33E-10	156	0.108							
	P31	WT	CRITICAL												
	F32G	WT	328.5	4.18E+05	1.33E-04	3.18E-10	68.3	0.0332		155.1	6.50E+05	3.70E-04	5.69E-10	36.2	0.0601
	A33	WT	CRITICAL												
	M34W	*WT*	*634.5*	*2.96E+05*	*1.15E-04*	*3.90E-10*	*119.6*	*0.0557*		*118.3*	*5.99E+05*	*2.36E-04*	*3.94E-10*	*24.2*	*0.226*
	S35H	WT	942.1	3.48E+05	1.32E-04	3.79E-10	202.8	0.0305							

			Sup / 25 °C							Purified / 37 °C					
CDR	Heavy chain - mutation	Light chain - mutation	Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)		Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
	S35Q	WT	587.6	3.75E+05	1.30E-04	3.46E-10	128.9	0.0834							
Heavy chain - CDR2	V48I	WT	182.6	4.60E+05	1.59E-04	3.46E-10	42.2	0.0224							
	V48Q	WT	49.5	6.54E+05	1.38E-04	2.10E-10	12.8	0.0418							
	A49T	WT	410.6	4.48E+05	1.51E-04	3.37E-10	96.2	0.0495							
	K50	WT	CRITICAL												
	I51R	WT	605.9	4.62E+05	7.60E-05	1.65E-10	132.1	0.168		126	6.79E+05	3.43E-04	5.05E-10	28.5	0.173
	S52	WT	CRITICAL												
	P53	WT	CRITICAL												
	G54	WT	CRITICAL												
	G55	WT	CRITICAL												
	S56R	WT	521.8	2.73E+05	1.55E-04	5.67E-10	97.4	0.0349							
	S56H	WT	582.3	3.56E+05	1.61E-04	4.52E-10	128.4	0.0384							
	W57F	WT	335.5	5.77E+05	1.28E-04	2.22E-10	78.1	0.0213		146.5	8.64E+05	4.53E-04	5.24E-10	37.2	0.0527

			Sup / 25 °C							Purified / 37 °C					
CDR	Heavy chain - mutation	Light chain - mutation	Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)		Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
	T58K	WT	553.5	4.60E+05	1.79E-04	3.90E-10	121	0.0168							
	T58R	WT	606.4	3.57E+05	1.52E-04	4.26E-10	131.5	0.0529							
	T58V	WT	621.7	8.03E+05	1.81E-04	2.26E-10	144	0.0386							
	Y59	WT	CRITICAL												
	Y60	WT	CRITICAL												
	S61R	WT	510.6	3.48E+05	1.62E-04	4.67E-10	119.1	0.0401							
	S61A	WT	546.2	3.79E+05	1.49E-04	3.94E-10	123.6	0.0376							
	D62Q	WT	1055.5	4.03E+05	1.48E-04	3.66E-10	237.3	0.0974		70.3	8.75E+05	6.01E-04	6.87E-10	17.5	0.156
	D62F	WT	279.2	4.20E+05	1.47E-04	3.50E-10	63.8	0.0214							
	T63D	WT	547.4	4.10E+05	1.74E-04	4.23E-10	127.4	0.0677							
	T63E	WT	622.5	3.80E+05	1.69E-04	4.45E-10	141.7	0.0419							

			Sup / 25 °C							Purified / 37 °C					
CDR	**Heavy chain - mutation**	**Light chain - mutation**	**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU)**	**Chi² (RU²)**		**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU)**	**Chi² (RU²)**
	T63K	WT	882.7	5.46E+05	2.12E-04	3.88E-10	137.4	0.134							
	T63G	WT	702.6	5.17E+05	2.09E-04	4.04E-10	134	0.0425							
	T63Q	WT	900.9	3.09E+05	1.84E-04	5.94E-10	208.1	0.261							
	V64A	WT	558.1	4.44E+05	1.78E-04	4.02E-10	130.3	0.0683							
	T65Y	WT	559.1	749000	0.000206	2.75E-10	123.8	0.0642							
	T65G	WT	3.2	597000	5.69E-05	9.54E-11	3	0.0498							
	G66E	WT	743	1.02E+06	1.10E-04	1.07E-10	176.8	0.242		105.2	1.91E+06	1.71E-04	8.95E-11	31.3	0.522
	G66D	*WT*	*783.5*	*1.30E+06*	*1.17E-04*	*9.02E-11*	*184.9*	*0.254*		*142.8*	*1.96E+06*	*1.36E-04*	*6.95E-11*	*39.9*	*0.513*
Heavy	A97V	WT	547.3	3.11E+05	1.79E-04	5.74E-10	128.2	0.0654							

			Sup / 25 °C							Purified / 37 °C					
CDR	Heavy chain - mutation	Light chain - mutation	Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)		Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
	R98G	WT	693.2	1.76E+05	2.51E-04	1.42E-09	148.9	0.344							
	Q99A	WT	879.2	3.68E+05	7.37E-04	2.00E-09	183.2	0.0575							
	Q99H	WT	1004.6	5.39E+05	1.05E-03	1.94E-09	213.9	0.094							
	L100Q	WT	1264.1	3.98E+05	1.53E-04	3.84E-10	281.2	0.0485		121.4	8.23E+05	4.10E-04	4.99E-10	28.4	0.155
	W101	WT	CRITICAL												
	G102D	WT	777.7	4.52E+05	6.00E-04	1.33E-09	136	0.0832							
	Y103D	WT	774.6	7.37E+05	2.19E-04	2.97E-10	140	0.0826							
	Y103S	WT	1016.4	5.46E+05	2.31E-04	4.23E-10	177.8	0.125							
	Y103E	WT	794.4	7.18E+05	1.65E-04	2.29E-10	192.4	0.0289							
	Y104V	WT	276.8	5.25E+05	7.03E-04	1.34E-09	41	0.0745							

			Sup / 25 °C							Purified / 37 °C					
CDR	Heavy chain - mutation	Light chain - mutation	Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)		Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
	A105Q	WT	524.4	4.74E+05	4.93E-04	1.04E-09	94.8	0.107							
	A105H	WT	520.8	4.04E+05	7.23E-04	1.79E-09	108	0.0869							
	L106F	WT	1138.8	5.91E+05	1.85E-04	3.14E-10	252.2	0.0955		444.9	8.52E+05	5.41E-04	6.34E-10	100.5	0.17
	L106I	WT	268.1	4.79E+05	1.34E-04	2.79E-10	60	0.0889							
	D107E	WT	9000.1	5.09E+05	1.47E-04	2.90E-10	200.7	0.0643		344.2	7.62E+05	4.02E-04	5.27E-10	78.7	0.145
	I108F	WT	1177.7	3.65E+05	1.46E-04	3.99E-10	251.7	0.0728		193.5	6.83E+05	4.01E-04	5.87E-10	42.7	0.216
	I108E	WT	1135.2	5.71E+05	1.74E-04	3.05E-10	254.4	0.156		234.8	9.21E+05	3.72E-04	4.04E-10	53.8	0.255
	I108S	WT	815.4	5.54E+05	1.41E-04	2.56E-10	177.3	0.0421		89.5	8.58E+05	3.36E-04	3.91E-10	20.8	0.362
Light chain	WT	S24P	44.3	4.84E+05	2.67E-06	5.51E-12	11.2	0.0502							

			Sup / 25 °C							Purified / 37 °C					
CDR	Heavy chain - mutation	Light chain - mutation	Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)		Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
	WT	S24K	739	3.11E+05	1.32E-04	4.24E-10	167.3	0.025							
	WT	S24V	787.2	3.40E+05	1.37E-04	4.03E-10	180.6	0.225							
	WT	S24I	597.7	4.76E+05	1.35E-04	2.84E-10	129.6	0.0541							
	WT	A25S	386.6	5.35E+05	1.05E-04	1.97E-10	90.6	0.0195		351.4	7.82E+05	3.42E-04	4.38E-10	86.1	0.21
	WT	A25T	513	5.08E+05	1.19E-04	2.35E-10	118	0.0222		77.5	8.89E+05	3.78E-04	4.25E-10	19.8	0.163
	WT	S26G	5.9	7.93E+05	2.23E-04	2.81E-10	3.6	0.15							
	WT	S26H	525.5	4.15E+05	1.49E-04	3.60E-10	117.4	0.111							
	WT	S26K	696.4	3.79E+05	1.64E-04	4.33E-10	155.4	0.144							
	WT	I27L	878.5	4.37E+05	1.45E-04	3.32E-10	202	0.0356		166.2	8.11E+05	4.49E-04	5.53E-10	39.1	0.18

			Sup / 25 °C							Purified / 37 °C					
CDR	Heavy chain - mutation	Light chain - mutation	Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)		Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
	WT	I27W	516.3	4.87E+05	1.61E-04	3.30E-10	113.3	0.129		151.7	1.06E+06	7.64E-04	7.19E-10	30.6	0.221
	WT	I27F	748.4	4.36E+05	1.93E-04	4.43E-10	168	0.0802		165.3	1.01E+06	6.90E-04	6.85E-10	38.5	0.186
	WT	S28E	673.8	4.20E+05	1.21E-04	2.89E-10	159.5	0.266							
	WT	S28R	468	3.69E+05	1.71E-04	4.64E-10	110.9	0.0196							
	WT	V29L	385.6	3.51E+05	1.71E-04	4.87E-10	83.4	0.171							
	WT	S30G	84.5	5.48E+05	1.73E-04	3.16E-10	19.3	0.0863							
	WT	S30A	760	2.55E+05	1.63E-04	6.39E-10	169	0.0653							
	WT	Y31G	405.9	3.05E+05	2.03E-04	6.66E-10	92.8	0.0183							
	WT	Y31F	745.1	2.92E+05	1.38E-04	4.72E-10	184.2	0.0274							

			Sup / 25 °C							Purified / 37 °C					
CDR	**Heavy chain - mutation**	**Light chain - mutation**	**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU)**	**Chi² (RU²)**		**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU)**	**Chi² (RU²)**
	WT	*M32L*	*563.2*	*5.91E+05*	*1.18E-04*	*1.99E-10*	*130*	*0.0192*		*188.7*	*8.85E+05*	*3.50E-04*	*3.95E-10*	*44.6*	*0.216*
	WT	M32V	426.5	5.05E+05	1.16E-04	2.30E-10	98.8	0.0226		175.6	8.61E+05	3.69E-04	4.29E-10	43.4	0.188
	WT	Y33	CRITICAL												
[redacted]															
Light chain - CDR2	WT	L45	CRITICAL												
	WT	L46V	486.4	4.60E+05	2.03E-04	4.42E-10	96.3	0.0311							
	WT	I47V	490	4.71E+05	1.95E-04	4.14E-10	93.3	0.0258							
	WT	Y48W	500.3	3.45E+05	1.23E-04	3.56E-10	121.7	0.0146							
	WT	D49E	376.2	4.59E+05	1.11E-04	2.41E-10	79.7	0.0725		164.3	6.68E+05	4.17E-04	6.24E-10	36.5	0.181
	WT	*M50D*	*544.3*	*8.06E+05*	*1.12E-04*	*1.38E-10*	*129.3*	*0.0328*		*48.4*	*1.49E+06*	*4.64E-04*	*3.11E-10*	*13.7*	*0.188*
	WT	S51K	817	3.96E+05	2.12E-04	5.35E-10	137.3	0.0479							
	WT	S51R	419.5	4.99E	2.31E-04	4.63E-10	64.6	0.0401							

			Sup / 25 °C							Purified / 37 °C					
CDR	**Heavy chain - mutation**	**Light chain - mutation**	**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU)**	**Chi² (RU²)**		**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU)**	**Chi² (RU²)**
				+05											
	WT	S51D	552.6	7.29E+05	2.16E-04	2.96E-10	77.5	0.0487							
	WT	*N52S*	*671.2*	*4.30E+05*	*1.52E-04*	*3.53E-10*	*160.2*	*0.0246*							
	WT	L53H	420.5	5.44E+05	2.18E-04	4.01E-10	64.9	0.0396							
	WT	L53W	10.2	1.19E+06	6.14E-06	5.18E-12	3.5	0.0526							
	WT	L53R	445.3	4.16E+05	1.85E-04	4.46E-10	87.7	0.0331							
	WT	A54G	302	4.75E+05	1.98E-04	4.16E-10	54.2	0.0241							
	WT	A54Y	104.2	6.52E+05	1.09E-04	1.68E-10	18	0.0725							
	WT	A54L	391.8	4.20E+05	2.10E-04	4.99E-10	68.2	0.0473							
	WT	S55W	472.5	5.63E+05	2.26E-04	4.01E-10	69.3	0.0716							

			Sup / 25 °C							Purified / 37 °C					
CDR	**Heavy chain - mutation**	**Light chain - mutation**	**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU)**	**Chi² (RU²)**		**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU)**	**Chi² (RU²)**
	WT	S55Y	615.7	4.36E+05	2.01E-04	4.61E-10	102.7	0.0384							
	WT	S55R	17.8	9.87E+05	1.07E-04	1.09E-10	5.2	0.0981							
	WT	S55L	661	6.68E+05	2.20E-04	3.29E-10	99.8	0.131							
Light chain - CDR3	WT	M88V	240.3	4.48E+05	1.07E-04	2.39E-10	49.4	0.0502		128.8	6.19E+05	4.41E-04	7.13E-10	29.8	0.176
	WT	M88A	328.8	4.51E+05	1.27E-04	2.82E-10	51.3	0.976		151.7	5.86E+05	1.32E-03	2.25E-09	27.5	0.141
	WT	Q89S	471.5	3.62E+05	2.72E-04	7.52E-10	61.2	0.0171							
	WT	Q89A	186.6	5.43E+05	2.27E-04	4.19E-10	32.2	0.085							
	WT	W90	CRITICAL												
	WT	S91A	345.3	3.41E+05	1.39E-04	4.07E-10	79	0.0325							
	WT	G92	CRITICAL												
	WT	Y93	CRITICAL												
	WT	P94	CRITICAL												

			Sup / 25 °C							Purified / 37 °C					
CDR	Heavy chain - mutation	Light chain - mutation	Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)		Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
	WT	Y95G	503.8	8.82E+05	3.38E-04	3.83E-10	51	0.126							
	WT	Y95I	274.3	5.93E+05	4.44E-03	7.48E-09	42.2	0.107							
	WT	T96L	399	5.20E+05	1.35E-04	2.59E-10	83.8	1.01		172.8	8.82E+05	7.95E-04	9.01E-10	37.1	0.234
	WT	T96V	523	4.54E+05	1.92E-04	4.23E-10	110.6	0.169		147.3	9.21E+05	6.39E-04	6.95E-10	34.8	0.202

[0556] *Biacore kinetics for anti-IL-6 single point mutations.* Data were obtained for samples prior (sup) at 25 °C and after purification at 37 °C. Mutations selected to be at final CDR combinations (Table 4) are italicized. CDR positions that did not tolerate mutations in the bacterial screening are indicated as critical.

TABLE 1.8

	Soup / 25 °C							Purified / 37 °C					
	Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)		Capture	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
Parent Sequence	981.4	3.15E+05	1.26E-04	3.99E-10	223.2	0.108		138.10	8.80E+05	1.39E-04	1.58E-10	34.6	0.397
Heavy Chain CDR2 G66D	783.5	1.30E+06	1.17E-04	9.02E-11	184.9	0.254		142.80	1.96E+06	1.36E-04	6.95E-11	39.9	0.513
M34I, G66D, I108V / M32L, M50D, N52S, M88Q	949.4	3.62E+05	2.00E-04	5.52E-10	212.2	0.0546		116.20	1.12E+06	7.02E-04	6.25E-10	28.2	0.37
M34I, G66D, I108V / M32L, M50A, N52S, M88Q	745.3	662000	0.000214	3.23E-10	155.7	0.0772		120	1150000	0.000757	6.56E-10	29.5	0.273
M34I, G66D, I108V / M32L, M50D, M88Q	1068	3.67E+05	2.15E-04	5.86E-10	244.5	0.19		132.20	1.21E+06	7.30E-04	6.02E-10	31	0.656
M34W, G66D, I108V / M32L, M50D, N52S, M88Q	603.5	4.67E+05	1.73E-04	3.70E-10	145.4	0.191		124.20	1.19E+06	5.91E-04	4.97E-10	30.1	0.625
M34W, G66D, I108V / M32L, M50A, N52S, M88Q	429.9	555000	0.000165	2.97E-10	100.9	0.0366		103.7	1080000	0.000662	6.15E-10	25.5	0.277
M34W, G66D, I108V / M32L, M50D, M88Q	509.4	7.44E+05	1.72E-04	2.31E-10	123.4	0.171		115.50	1.34E+06	6.27E-04	4.69E-10	29.9	0.407

[0557] *Biacore kinetics data for anti-IL-6 CDR variants.* Kinetic data were obtained from cell culture supernatants (sup) at 25 °C and purified samples at 37 °C. Heavy and light chain CDR mutations are indicated on the left and right side, respectively (heavy/light). Capture, kon (ka), koff (kd) rates and dissociation constants are listed in the table.

TABLE 1.9. ANTI-IL-6 HEAVY CHAIN VARIABLE REGION SEQUENCES. SOME EMBODIMENTS OF CDRS ARE UNDERLINED.

ID	Sequence
I	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAISWVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRFTFS LDTSKSTAYLQMNSLRAEDTAVYYCARQLWGYYALDVWGQGTLVTVSS (SEQ ID NO: 140)
II	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAWSWVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCARQLWGYYALDVWGQGTLVTVSS (SEQ ID NO: 141)

TABLE 1.10. ANTI-IL-6 LIGHT CHAIN VARIABLE REGION SEQUENCES. SOME EMBODIMENTS OF CDRS ARE UNDERLINED.

ID	Sequence
III	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQQKPGKAPKLLIYDDSSLASGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK (SEQ ID NO: 142)
IV	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQQKPGKAPKLLIYDASSLASGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK (SEQ ID NO: 143)
V	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQQKPGKAPKLLIYDDSNLASGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK (SEQ ID NO: 144)

EXAMPLE 2

ANTI-IL-6 AB VEGF TRAP MOLECULE DESIGN AND CONSTRUCTION

[0558] The VEGF binding moiety of the IL-6Ab-VEGF Trap consisted of a fusion between VEGFR1 domain 2 and VEGFR2 domain 3 (Table 2.0). This fusion works as a VEGF trap, preventing VEGF from binding to cellular VEGF receptors.

TABLE 2.0. VEGF TRAP SEQUENCE

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEAT VNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQS GSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK (SEQ ID NO: 145)

[0559] 12 constructs were tested containing the VEGF trap in two distinct configurations (Table 2.1). In the first configuration, the VEGF trap was positioned at the beginning of the protein, followed by a duplicate series of a Gly-Gly-Gly-Gly-Ser linker (GS),

which connects the VEGF trap to the N-terminus of the anti-IL-6 heavy chain (VEGF Trap-AntiIL-6; Table 2.1, molecules A-F). In the second configuration, the variable and constant domains of the heavy chain (Fab region) are connected to the VEGF Trap via the GS linker, and then the Fc domain is fused to the C-terminal end of the VEGF trap. Thus, in this configuration, the VEGF trap is sandwiched between the antibody Fab and Fc regions (AntiIL-6-VEGF Trap; Table 2.1, molecules G-L).

TABLE 2.1. HEAVY AND LIGHT CHAIN SEQUENCES FOR DUAL INHIBITOR MOLECULES. SOME EMBODIMENTS OF CDRS ARE UNDERLINED IN THE HEAVY AND LIGHT CHAINS, VEGF TRAP SEQUENCE IS BOLDED, GLY-SER LINKER IS ITALICIZED (AND BOLDED).

ID	Heavy chain	Light chain
A	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK*GGGGSGGGGS*** EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAISWVRQAPGKGLEWVAKISPGGS WTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAED TAVYYCARQLWGYYALDVWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 269)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 146)
B	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK*GGGGSGGGGS*** EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAISWVRQAPGKGLEWVAKISPGGS WTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAED TAVYYCARQLWGYYALDVWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDASSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 148)

ID	Heavy chain	Light chain
	LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 147)	
C	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK*GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAISWVRQAPGKGLEWVAKISPGGS WTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAED TAVYYCARQLWGYYALDVWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 149)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 150)
D	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK*GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAWSWVRQAPGKGLEWVAKISPGG SWTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAE DTAVYYCARQLWGYYALDVWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSCSPGK (SEQ ID NO: 151)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 152)
E	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK*GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAWSWVRQAPGKGLEWVAKISPGG SWTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAE DTAVYYCARQLWGYYALDVWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDASSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 154)

ID	Heavy chain	Light chain
	SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSCSPGK (SEQ ID NO: 153)	
F	**SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFN WEYPSSKHQHKKLVNRDLKTQSGSEMKKFLST LTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRV HEK*GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGFTFSPFAWSWVRQAPGKGLEWVAKISPGG SWTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAE DTAVYYCARQLWGYYALDVWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSCSPGK (SEQ ID NO: 155)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 156)
G	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAIS WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQLWG YYALDVWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC*GGGGSGGGGS***SDTGRPFVE MYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKF PLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTC EATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIE LSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQ HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSD QGLYTCAASSGLMTKKNSTFVRVHEK**DKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 157)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 158)
H	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAIS WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQLWG YYALDVWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC*GGGGSGGGGS***SDTGRPFVE MYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKF PLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTC EATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIE LSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQ HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSD QGLYTCAASSGLMTKKNSTFVRVHEK**DKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDASSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 160)

ID	Heavy chain	Light chain
	LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 159)	
I	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAIS WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQLWG YYALDVWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC***GGGGSGGGGS*****SDTGRPFVE** **MYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKF** **PLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTC** **EATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIE** **LSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQ** **HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSD** **QGLYTCAASSGLMTKKNSTFVRVHEK**DKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 161)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 162)
J	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAWS WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQLWG YYALDVWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC***GGGGSGGGGS*****SDTGRPFVE** **MYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKF** **PLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTC** **EATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIE** **LSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQ** **HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSD** **QGLYTCAASSGLMTKKNSTFVRVHEK**DKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 163)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 164)
K	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAWS WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQLWG YYALDVWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC***GGGGSGGGGS*****SDTGRPFVE** **MYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKF** **PLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTC** **EATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIE** **LSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQ** **HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSD** **QGLYTCAASSGLMTKKNSTFVRVHEK**DKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDASSLASGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC (SEQ ID NO: 166)

ID	Heavy chain	Light chain
	LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 165)	
L	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAWS WVRQAPGKGLEWVAKISPGGSWTYYSDTVTDRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCARQLWG YYALDVWGQGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC***GGGGSGGGGS*****SDTGRPFVE** **MYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKF** **PLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTC** **EATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIE** **LSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQ** **HKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSD** **QGLYTCAASSGLMTKKNSTFVRVHEK**DKTHTC PPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSCSPGK (SEQ ID NO: 167)	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQ QKPGKAPKLLIYDDSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 168)

[0560] Biacore (setup as described in the previous session) kinetic data showed that although the K_D values among all variants are comparable, there is a variation in the capture values for the supernatant samples (Table 2.2). This parameter is sensitive to the amount of antibody present in solution, which suggested a variation on the level protein expression among these molecules due to the different CDR compositions. On the other hand, the two VEGF Trap positions tested did not show marked influence on the kinetic data. Experiments monitoring expression levels (data not shown) showed the IL-6-VEGF Trap configuration has about 20% higher yield of expression in comparison to VEGF Trap-AntiIL-6 configuration. Preliminary Biacore data also indicates AntiIL-6-VEGF Trap configuration binds tighter to a VEGF homologue, the placental growth factor (PLGF) (data not shown).

TABLE 2.2.

[0561] Biacore kinetics data for dual inhibitor variants A-L binding to IL-6. Kinetic data were obtained from cell culture supernatants (sup) samples at 25 °C and purified samples at 37 °C. Capture, kon (ka), koff (kd) rates and dissociation constants are listed.

	Sup / 25 °C				Purified / 37 °C			
ID	**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**
A	521.4	3.09E+05	1.82E-04	5.89E-10	100	1.11E+06	5.38E-04	4.84E-10

	Sup / 25 °C				Purified / 37 °C			
ID	**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Capture**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**
B	144.3	6.05E+05	1.85E-04	3.05E-10	106.6	1.06E+06	5.36E-04	5.07E-10
C	474.6	3.79E+05	1.50E-04	3.94E-10	92	1.01E+06	4.81E-04	4.77E-10
D	356.2	4.33E+05	1.09E-04	2.53E-10	105.4	1.23E+06	4.47E-04	3.65E-10
E	228	5.04E+05	1.51E-04	2.99E-10	97.9	1.08E+06	4.59E-04	4.24E-10
F	272.1	6.11E+05	1.37E-04	2.25E-10	93.9	1.31E+06	4.46E-04	3.4E-10
G	519	2.52E+05	1.49E-04	5.89E-10	109	1.26E+06	5.67E-04	4.51E-10
H	424.7	4.54E+05	1.62E-04	3.56E-10	97.3	1.34E+06	5.29E-04	3.96E-10
I	552.8	2.17E+05	1.47E-04	6.79E-10	108.9	1.15E+06	4.99E-04	4.35E-10
J	335	5.34E+05	1.49E-04	2.80E-10	88.9	1.42E+06	6.64E-04	4.68E-10
K	279.8	6.29E+05	1.78E-04	2.82E-10	93.9	1.28E+06	5.79E-04	4.52E-10
L	312.4	7.05E+05	1.69E-04	2.39E-10	91.9	1.49E+06	6.50E-04	4.36E-10

EXAMPLE 3

Dual inhibitor binding to VEGF via Biacore

[0562] To evaluate the binding kinetics of the different dual inhibitors configurations to VEGF, a series of Biacore assays were performed to measure the affinity between these molecules. Binding kinetics were measured at 37 °C in buffer HBS-EP+ containing 1 mg/mL BSA. Anti-VEGF agents (VEGF Trap-AntiIL-6 (5 µg/mL), AntiIL-6-VEGF Trap (5 µg/mL), OG1950 (1 µg/mL) and Eylea (0.5 µg/mL)) were captured on a Protein A chip (GE) at 10 µL/min for 25 seconds, five concentrations of VEGF-A165 (0.19, 0.56, 1.67, 5, 15 nM) were flowed over captured antibodies for 120 seconds at 30 µL/min and dissociated for 30 min. The sensor chip surface was regenerated by 60 seconds injection of 10 mM Glycine, pH 1.7 at a flow rate of 50 µl/min. All sensorgrams were double reference subtracted and fit using a 1:1 Langmuir binding model.

[0563] Data show VEGF trap component in the dual inhibitor molecule binds tightly to VEGF-A independent of configuration. The anti-IL-6 molecule component did not seem to influence VEGF-A binding to the VEGF trap, since the dual inhibitor molecules show similar affinity to VEGF affinity as another VEGF trap-based molecule (Eylea). (Figure 7)

IL-6 and VEGF-A binding to dual inhibitor molecules

[0564] A Biacore assay was implemented to directly monitor the dual binding of IL-6 and VEGF-A. Dual inhibitor molecules (2 µg/mL) were captured on a Protein A chip (GE) at

10 µL/min for 25 seconds, five concentrations of IL-6 and/or VEGF-A165 (0.19, 0.56, 1.67, 5, 15 nM) were flowed individually or mixed over captured antibodies for 120 seconds at 30 µL/min and dissociated for 30 min. The sensor chip surface was regenerated by 60 seconds injection of 10 mM Glycine, pH 1.7 at a flow rate of 50 µl/min. All data were double reference subtracted, and the sum of individual IL-6 and VEGF-A sensorgrams were used to create a theoretical curve, which was compared to the sensorgrams of mixed samples.

[0565] Results demonstrated that theoretical and experimental curves are nearly superimposed, which strongly supports a model where both targets bind to the dual inhibitor molecules without affecting their respective interactions. (Figure 8)

Anti-IL-6 binding to IL-6/IL-6-R complex ELISA

[0566] IL-6/IL-6R complexes can bind to the membrane bound receptor, gp130, and this trimeric complex stimulates IL-6 signaling, such as proliferation and upregulation of VEGF. Inhibition of either IL-6 binding to IL-6R or IL-6/IL-6R binding to gp130 blocks IL-6 mediated downstream signaling pathways. To determine whether the anti-IL-6 antibodies block either of these binding events, an ELISA was first established to assess whether they can bind to IL-6 while it is complexed with IL-6 receptor.

[0567] A Nunc MaxiSorp ELISA was coated with 100 µl of 1 µg/ml of recombinant human IL-6R (R&D Systems) overnight. 10 nM IL-6 (R&D Systems) was mixed with a 3-fold dilution series of anti-IL-6 (final concentrations of 30 nM down to 10 pM). After blocking and washing, the mixture was added to the IL-6R coated plates. This assay was also performed by first incubating the IL-6R coated plate with 10 nM IL-6, washing, and then adding the anti-IL-6 antibody. For both methods, bound antibody/IL-6 complexes were detected with an anti-Human IgG HRP antibody and developed with TMB (KPL) for 10 minutes. Absorbance at 450 nM was measured on a SpectraMax Plus plate reader (Molecular Devices) and plotted against the concentration of antibody using GraphPad Prism software and fit with a non-linear regression curve.

[0568] A Nunc MaxiSorp ELISA was coated with 100 µl of 1 µg/ml of recombinant human IL-6 (R&D systems) overnight. A 3-fold dilution series of anti-IL-6 (R&D Systems; final concentrations of 30 nM down to 10 pM) was performed and solutions were added to the blocked

IL-6 coated plates. Antibody/IL-6 complexes were detected with an anti-Human IgG HRP antibody and developed with TMB (KPL) for 10 minutes. Absorbance at 450 nM was measured on a SpectraMax Plus plate reader (Molecular Devices) and plotted against the concentration of antibody using GraphPad Prism software and fit with a non-linear regression curve.

[0569] Under the conditions tested, the anti-IL-6 antibody described here binds specifically to IL-6, but not when it is complexed with IL-6 receptor, suggesting that the antibody and IL-6R share a common epitope on IL-6. The results are shown in FIG. 4.

Competitive IL-6/IL-6R ELISA

[0570] An ELISA to determine whether the anti-IL-6 antibody and dual IL-6/VEGF inhibitors blocked IL-6 from binding to IL-6R was then performed

[0571] A Nunc MaxiSorp ELISA was coated with 100 µl of 1 µg/ml of anti-IL-6/IL-6R complex antibody (R&D Systems; IL-6/IL-6R DuoSet) overnight. The plate was blocked in 2% bovine serum albumin (BSA) in PBS-Tween (PBST) for 2 hours. During blocking, 6 nM of IL-6 (R&D Systems), 1 nM of IL-6R (R&D Systems), and an 8-point, 3-fold dilution series of dual inhibitors or single arm controls (starting with 30 nM) were incubated at room temperature. This mixture was then added to the ELISA plate following a wash step. After 1 hour, the plate was washed and incubated with biotinylated anti-IL-6/IL-6R antibody (R&D Duoset). IL-6/IL-6R complexes were detected with steptravidin-HRP (1:2000 dilution; R&D Systems) and developed using TMB reagent (KPL) for 10 minutes. Absorbance at 450 nM was measured on a SpectraMax Plus plate reader (Molecular Devices) and plotted against the concentration of antibody using GraphPad Prism software and fit with a non-linear regression curve.

[0572] Under these conditions, the anti-IL-6 antibody and dual IL-6/VEGF inhibitors effectively compete with IL-6R for binding to IL-6, and therefore inhibit the IL-6/IL-6R interaction. The IC50 values for dual inhibitor molecules are comparable (anti-IL-6 = 0.36 nM, VEGF Trap-anti-IL-6 = 0.47 nM, and anti-IL-6-VEGF Trap = 0.32 nM), indicating that the configuration of the molecule does not influence IL-6 binding or inhibition. Eylea served as a negative control. (Figure 9)

Competitive VEGF/VEGFR ELISA

[0573] To assess the function of the VEGF trap, the ability of the dual IL-6/VEGF inhibitors to block VEGF binding to its receptor (VEGFR) was tested by a competitive ELISA.

[0574] A Nunc MaxiSorp ELISA was coated with 100 µl of 1 µg/ml of recombinant, human VEGFR-Fc (R&D Systems) overnight. The plate was blocked in 2% bovine serum albumin (BSA) in PBS-Tween (PBST) for 2 hours. During blocking, 100 pM biotinylated VEGF (btVEGF; R&D Systems), was incubated with increasing concentrations of anti-IL-6-VEGF Trap, VEGF Trap-anti-IL-6, or the bivalent anti-VEGF antibody OG1950. Bound bt-VEGF was detected with steptravidin-HRP (1:2000 dilution; R&D Systems) and developed using TMB reagent (KPL) for 10 minutes. Absorbance at 450 nM was measured on a SpectraMax Plus plate reader (Molecular Devices) and plotted against the concentration of antibody using GraphPad Prism software and fit with a non-linear regression curve.

[0575] Under these conditions, the dual IL-6/VEGF inhibitors bind to VEGF and inhibits it from binding to its receptor. When the VEGF trap is situated at the N-terminal end of the anti-IL-6 antibody, VEGF inhibition is roughly 2.5-fold more potent than the molecule whose VEGF trap is positioned between the Fab and Fc domains of the anti-IL-6 antibody (IC50 1.74 nM vs. 4.53 nM, respectively), likely due to a change in valency from 2 binding sites to 1 binding site. The results are shown in FIG. 9.

EXAMPLE 4

IL-6-VEGF Bridging ELISA

[0576] To ensure that the dual inhibitors can simultaneously bind to both IL-6 and VEGF, they were subjected to a bridging ELISA where they first interact with plate-bound IL-6, and then are incubated with biotinylated VEGF (btVEGF). Detection of btVEGF by streptavidin HRP indicates that IL-6 bound molecules are also able to bind btVEGF.

[0577] A Nunc MaxiSorp ELISA was coated with 100 µl of 1 µg/ml of recombinant, human IL-6 (Tonbo Sciences) overnight. The plate was blocked in 2% bovine serum albumin (BSA) in PBS-Tween (PBST) for 2 hours. During blocking, 100 pM btVEGF) was incubated with

increasing concentrations of anti-IL-6-VEGF Trap, VEGF Trap-anti-IL-6, or the bivalent antiVEGF antibody OG1950 as a negative control. Bound btVEGF was detected with steptravidin-HRP (1:2000 dilution; R&D systems) and developed using TMB reagent (KPL) for 10 minutes. Absorbance at 450 nM was measured on a SpectraMax Plus plate reader (Molecular Devices) and plotted against the concentration of antibody using GraphPad Prism software and fit with a non-linear regression curve.

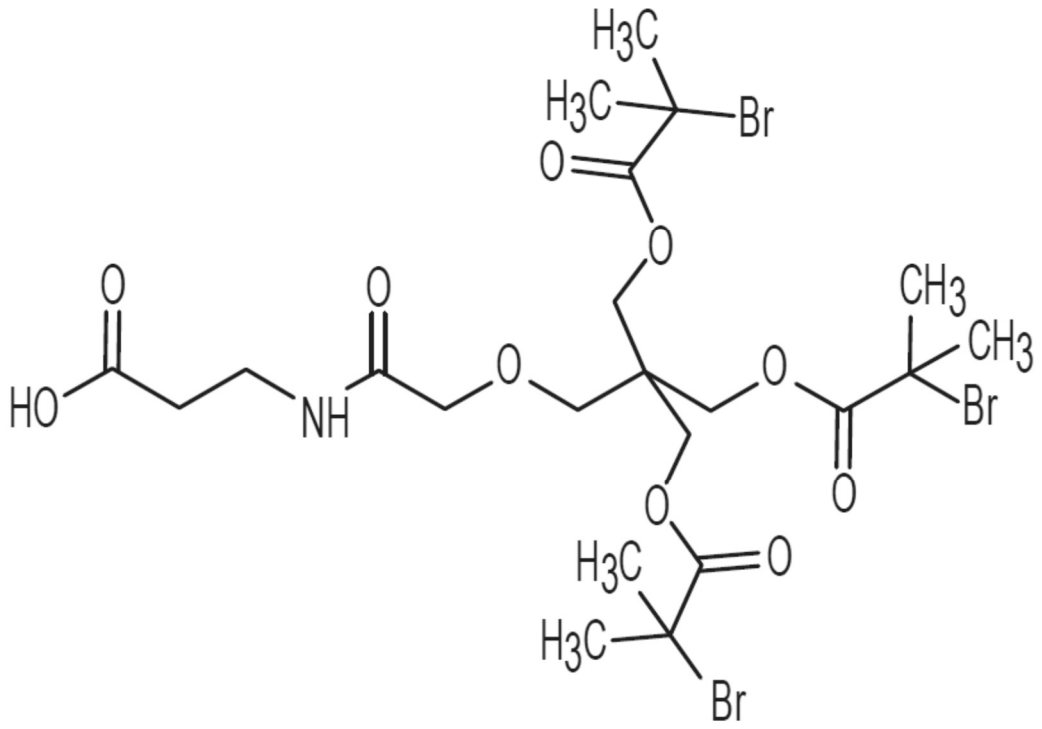
[0578] This assay confirmed that the dual IL-6/VEGF inhibitors simultaneously bind IL-6 and btVEGF. The orientation of the anti-IL-6 and VEGF Trap has slightly variable binding, with the anti-IL-6-VEGF Trap orientation being more favorable under these conditions (EC50 VEGF Trap-antiIL-6 = 0.079 nM and antiIL-6-VEGF Trap = 0.026 nM). Eylea and anti-IL-6 served as negative controls. The results are shown in FIG. 9.

EXAMPLE 5

Route 1 Synthesis of OG1802.

[0579] A first route for the synthesis of OG1802 is as follows. First, TFA/amine salt initiator (Compound L) having the structure shown in FIG. 2A was synthesized as follows.

[0580] First, Compound K, having the structure shown in FIG. 2B was synthesized as follows. Into a 200 mL round bottom flask under nitrogen was placed Compound J (OG1563) (1.9 g, 2.67 mmol, 3.3 equiv)



COMPOUND J

and Compound E (0.525 g, 0.81 mmol, 1.0 equiv) (see FIG. 2L) followed by dimethylformamide (10 mL) then diisopropylethylamine (2.5 mL, 14.6 mmol, 18 equiv). The flask was cooled to 0°C using an ice bath. To this was added propylphosphonic anhydride solution (50 wt. % in ethyl acetate, 2.5 mL, 4.04 mmol, 5 equiv) over ~6 minutes.

[0581] The reaction was warmed to room temperature and stirred for 15 minutes. The reaction was quenched by adding water (20 mL), saturated aqueous sodium bicarbonate (20 mL) and ethyl acetate (100 mL). The organic layer was separated and the aqueous layer extracted with ethyl acetate (75 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate (30 mL), 0.5 M aqueous citric acid (40 mL), water (25 mL), and saturated aqueous sodium chloride (40 mL), then dried (sodium sulfate), filtered and concentrated under vacuum. The residue which was used without further purification resulted in 2.0 g (0.80 mmol, 99%) of Compound K.

1H NMR (400 MHz DMSO-d6): δ = 1.36 (s, 9H, OCCH3), 1.90 (s, 54H, CC(CH3)2Br), 2.31 (t, J = 7.2 Hz, 6H, CCH2CH2NH), 2.98 (d, J = 5.6 Hz, 6H, CCH2NH), 3.04 (q, J = 6.0 Hz, 2H, OCH2CH2NH), 3.18 (s, 2H, OCH2C), 3.3-3.37 (m, 8H, CH2), 3.47-3.55 (m, 12H, CH2), 3.58 (s,

6H, OCH2C), 3.87 (s, 6H, O=CCH2O), 4.27 (s, 18H, CCH2OC=O), 6.74 (br t, 1H, CH2NHC=O), 7.69 (t, J = 6.8 Hz, 3H, CH2NHC=O), 7.84 (t, J = 6.0 Hz, 3H, CH2NHC=O).
LC-MS (ES, m/z): [(M+2H-boc)/2]+ Calcd for (C84H136Br9N7O33+2H-Boc)/2 = 1196.6; Found 1196.6.

[0582] Next Compound L (FIG. 2A) was synthesized as follows: into a 100 mL round bottom under nitrogen was added Compound K (2.0 g, 0.8 mmol), dichloromethane (10 mL) followed by trifluoroacetic acid (5 mL). The reaction was stirred at room temperature for 30 minutes. The reaction was concentrated under a vacuum. The reaction was diluted using dichloromethane (10 mL) and concentrated under a vacuum. The residue was dissolved using acetonitrile (10 mL), filtered through a syringe filter (Acrodisc CR25, PN 4225T) and loaded onto a preparatory HPLC column and eluted with 60% acetonitrile in water (with 0.1% trifluoroacetic acid) up to 98% acetonitrile (with 0.1% trifluoroacetic acid). The tubes containing product were pooled, concentrated under vacuum, frozen and placed on a lyophilizer. This resulted in 990 mgs (0.4 mmol, 50% over 2 steps) Compound L as a white powder.
1H NMR (400 MHz DMSO-d6): δ = 1.90 (s, 54H, CC(CH3)2Br), 2.31 (t, J = 7.2 Hz, 6H, CCH2CH2NH), 2.97-3.0 (m, 8H, CCH2NH and OCH2CH2NH), 3.17 (s, 2H, OCH2C), 3.3 (q, 6H, CH2CH2NHC=O), 3.4-3.59 (m, 20H, CH2), 3.87 (s, 6H, O=CCH2O), 4.27 (s, 18H, CCH2OC=O), 7.69-7.84 (m, 9H, both CH2NHC=O and NH3+).
LC-MS (ES, m/z): [(M+2H)/2]+ Calcd for (C84H136Br9N7O33+2H)/2 = 1196.6; Found 1197.4.

[0583] Next, Compound L (FIG. 2A) was used as an initiator to synthesize MPC polymer. Initiator is typically prepared as a stock solution in DMF of about 100 mg/mL. The initiator and the ligand (2,2'-bipyridyl) were introduced into a Schlenk tube. The resultant solution was cooled to -78°C using a dry ice/acetone mixture, and was degassed under vacuum for 10min. The tube was refilled under Argon and the catalyst (CuBr unless otherwise indicated), kept under Argon**,** was introduced into the Schlenck tube (the Molar ratio of atom bromine on the initiator/catalyst (CuBr)/ligand was kept at 1/1/2). The solution became dark brown immediately. The Schlenk tube was sealed and immediately purged by applying **a** short cycle vacuum/Argon. A solution of HEMA-PC was prepared by mixing a defined quantity of monomer, prepared in a glovebox kept under nitrogen, with 200 proof degassed ethanol. The monomer solution was added drop wise into the Schlenk tube (via cannula) (and homogenized by light stirring)**.** The temperature was maintained at -78°C. A thorough vacuum was applied to the

reaction mixture for at least 10 to 15 min. until bubbling from the solution ceased. The tube was then refilled with Argon and warmed to room temperature. The solution was stirred, and as the polymerization proceeded, the solution became viscous. After 3 to 8 hours or just left overnight, the reaction was quenched by direct exposure to air in order to oxidize Cu (I) to Cu (II), the mixture became blue-green in color, and was passed through a silica column in order to remove the copper catalyst. The collected solution was concentrated by rotary evaporation and the resulting mixture was either precipitated with tetrahydrofuran or dialyzed against water followed by freeze drying to yield a free-flowing white powder. Table 5.0 below sets forth polymer data for polymer employing compound L as an initiator.

TABLE 5.0

Theor. MW (kDa)	Polymer ID No.	Initiator	Mn(kDa)	Mp(kDa)	PDI
500	130	L	490	530	1.1
750	150	L	645	750	1.1

[0584] Next, the maleimide Mal-PEG4-PFP ester was snapped on (as set forth in FIG. 2C) to the 750 kDa polymer referred to above to provide OG1802. Into a 20 mL vial was placed Polymer R3707 (750 kDa polymer made using L as initiator, 515mg) and dissolved using ethanol (4.0mL) after stirring for 40 minutes. To this was added a 1% solution of 4-methylmorpholine in acetonitrile (22uL). In a separate vial was dissolved Mal-PEG4-PFP (1.97mg) in acetonitrile (1.0mL) and this solution was added to the polymer solution over ~2 minute at room temperature and the resulting solution was stirred for overnight. The reaction was diluted with 0.1% aqueous trifluoroacetic acid (2 mL) (pH ~5) followed by water (~12 mL), filtered through a syringe filter (Acrodisc Supor, PN 4612) and placed evenly into 3 Amicon centrifuge membrane dialysis tubes (30,000 mwco). The tubes were diluted and mixed with water (~5 mL each), placed into centrifuge (rpm 3200) for 25 minutes. The filtrate is removed for analysis while the retentate is diluted and mixed with water (~10 mL/tube). The centrifuge procedure repeated 5 more times, after which the retentate is removed and placed into a vial. The Amicon membrane tubes were rinsed with water (2 x ~2 mL each tube) and this combined with the retentate. The retentate solution was

filtered through a syringe filter (Acrodisc Supor, PN 4612), frozen and placed on a lyophilizer. This resulted in 485 mgs as a white powder.

Example 6 - Synthesis of Initiator OG1786

[0585] OG1786 is the nine-arm initiator for polymer synthesis used as a precursor in the synthesis of OG1802. Each arm is terminated with a 2-bromoisobutyrate which is capable of initiating polymerization under ATRP. OG1786 is a salt of trifluoro acetic acid (TFA) as shown in FIG. 2D. OG1786 is prepared as follows. First, OG1550 is reacted with TFA (trifluoro acetic acid) to produce OG1546 as depicted in FIG. 2E.

[0586] In a 1L round bottom flask equipped with a magnetic stir bar and an addition funnel was added OG1550 (14.8 g), methyl tert-butyl ether (MTBE) (350 ml) and water (30 ml). The mixture was stirred to dissolve the OG1550, then cooled in an ice bath. To this mixture was added a solution of trifluoroacetic acid (4.9 ml) in water (90 ml) dropwise over 90 minutes. After addition is complete the mixture was stirred an additional 15 minutes then removed from the ice bath and allowed to warm to room temperature. The mixture was stirred (after removal from the ice bath) for a further 4-5 hours, until tlc showed ~5% starting material remaining, and the pH of the aqueous was between 3 and 4 (pH paper).

[0587] The mixture was partitioned. The MTBE layer was washed with water (30 ml). Combine aqueous layers then the aqueous extracted with MTBE (150 ml). This second MTBE phase was washed with water (30 ml). The combined aqueous layers were washed with a third portion of MTBE (100 ml). The third MBTE phase was washed with water (25 ml). The aqueous layers were again combined (~250 ml, pH ~4, by pH paper).

[0588] The product was collected by lyophilization. 11.5 g white solid was obtained. This material is extremely hygroscopic, so best handled under nitrogen. The product was confirmed by LCMS.

[0589] The prepared OG1546 was then reacted with OG1563 to yield OG1784 (as depicted in FIG. 2F).

[0590] In a 250 ml flask under nitrogen equipped with a stir bar was added OG1546 (hygroscopic, 9.0 g), followed by N,N-dimethylformamide (110 ml). The mixture was stirred at room temperature until all OG1546 dissolved (about 15 minutes), then OG1563 (29.9 g) was added, and the mixture stirred a further 3 minutes until the OG1563 had also been dissolved. The resulting solution was cooled in an ice bath, and N,N-diisopropylethylamine (37.6 ml) was added

over 3 minutes, followed by propylphosphonic anhydride (T3P), 50% in ethyl acetate (34.5 ml) dropwise over 5 minutes (T3P addition is exothermic). After T3P addition was complete, the flask was removed from the cooling bath and allowed to reach room temperature. Samples were then taken at 5 minute intervals for LCMS analysis. The reaction showed very light yellow/tan color.

[0591] After 20 minutes the reaction was cooled again in an ice bath and 5 ml water added. The mixture was then removed from the cooling bath and a further 50 ml water portion added, followed by 50 ml 0.5 M citric acid then isopropylacetate (300 ml). The mixture was partitioned. The aqueous phase (~300 ml) was extracted with additional isopropyl acetate (150 ml). The aqueous phase was AQ1 for HPLC test. The combined organics were washed with aqueous citric acid (115 ml, 65 mM, which was the mixture of 15 ml of 0.5 M citric acid plus 100 ml water), and the aqueous phase was AQ2 (pH~3). The organic phase was washed with water/saturated sodium chloride (100 ml/25 ml), and the aqueous phase was AQ3 (pH~3). The organic phase was finally washed with saturated sodium chloride (100 ml), and the aqueous phase was AQ4. None of the AQ fractions contained any significant product (data not provided). The organic phase confirmed the product via LCMS. The product was dried over sodium sulfate (80 g), filtered and rinsed with isopropyl acetate (75 ml), and concentrated on a rotary evaporator to a tan oil (33.2 g). The crude was stored overnight under nitrogen.

[0592] The next day the crude was allowed to come to room temperature, then dissolved in acetonitrile/water (46 ml/12 ml) and filtered using an HPLC filter disk (Cole-Parmer PTFE 0.2 µm, product number 02915-20). The filtrate was split into three equal portions and purified in three runs.

[0593] The filtrate was loaded onto a RediSep Rf Gold C18 column (275 g, SN 69-2203-339, Lot# 24126-611Y) equilibrated with 50% acetonitrile/water. The material was eluted at 100 ml/min using the following gradient (solvent A: water, solvent B: acetonitrile). All the relevant fractions were checked by HPLC. The fractions adjudged to be pure enough were pooled (from all three runs) and concentrated (bath temperature kept at about 20°C) on rotovap, then partitioned between dichloromethane (100 ml) and water (5 ml)/saturated sodium chloride (25 ml). The aqueous was extracted twice more with dichloromethane (2 x 30 ml). The combined organics were dried over sodium sulfate (35 g), filtered, rinsed with DCM (30 ml), and concentrated. The product and purity were confirmed by LCMS methods. The isolated yield and the purity of the R5172 and R5228 lots are shown in Table 6.0.

TABLE 6.0

OG1784 lot	R5172	R5228
OG1546 used	5.3 g	9.0 g
OG1563 used	17.6 g	29.9 g
Isolated yield	53%	58%
Purity (a/a 210 nm)	99.3%	100.0%

[0594] Next OG1405 was prepared from OG1784 as depicted in FIG. 2G. In a 500 ml round bottom flask equipped with a magnetic stir bar was added OG1784 (20.9 g), followed by dichloromethane (50 ml) then trifluoroacetic acid (20 ml). The mixture was stirred at room temperature and HPLC analysis showed complete deprotection in 23 minutes. The mixture was concentrated on a rotary evaporator, redissolved in dichloromethane (25 ml) and re-concentrated, then redissolved in acetonitrile (25 ml) and re-concentrated. The product was confirmed by LCMS. The material from above (OG1405, 34.5 g, assume 21.0 g as quantitative yield) was used as a crude oil in the next step. No purification is needed.

[0595] Next, OG1405 was reacted with OG1402 to prepare OG1785 as set forth in FIG. 2H. In a 500 ml flask under nitrogen equipped with a stir bar was placed OG1402 (5.5 g), followed by acetonitrile (70 ml), then N,N-diisopropylethylamine (26.3 ml) and T3P solution (see above) (7.9 ml). The solution was stirred at room temperature for 30 minutes, then cooled in an ice water bath and a solution of OG1405 (crude oil from above, 34.5 g) in acetonitrile (70 ml) added. The mixture was warmed to room temperature. After 20 minutes the reaction was cooled in an ice water bath and quenched with water (5 ml). The mixture was then concentrated under vacuum using a rotary evaporator to half volume. Samples were taken for LCMS.

[0596] More water (50 ml), followed by 0.5 M citric acid (75 ml) and isopropyl acetate (175 ml) was added. The mixture was partitioned in 5 minutes. The aqueous was extracted with additional isopropyl acetate (50mL). The combined organics were washed with aqueous citric acid (0.13 M, 30 ml, consist of 10 ml of 0.5 M citric acid and 20 ml water). The organics were then washed with the mixture of saturated sodium chloride (25 ml) and water (25 ml), then finally washed with the saturated sodium chloride (25 ml). They were then dried over sodium sulfate

(124 g), filtered and rinsed with isopropyl acetate (30 ml), and concentrated under rotary evaporator to a tan oil (27.3 g). Samples were taken for LCMS analysis.

[0597] The oil was dissolved in acetonitrile/water (3:1, 15 ml/5ml), filtered through an HPLC filter disk (Cole-Parmer PTFE membrane 0.2 µm, product number 02915-20) and split into three equal portions, each of which were individually purified as follows.

[0598] Portions were loaded onto Redi-Sep Gold C18 column (275 g, SN – 69-2203-339, Lot 241234-611W) equilibrated at 50% solvent B (acetonitrile)/50% solvent A (water). The material was then purified by reverse phase HPLC with a solvent A: water/solvent B: acetonitrile gradient. Appropriate fractions were pooled and partitioned between dichloromethane (150 ml) and water (5 ml)/saturated sodium chloride (25 ml). The aqueous was extracted twice with dichloromethane (2 x 50 ml). Combined organics were dried over sodium sulfate (60g), filtered and rinsed with dichloromethane (40 ml) and concentrated. Structure and purity were confirmed by various analytics including LCMS: OG1785 was isolated as a foamy solid (R5329, 19.0 g, 83% yield, 95.1% purity (a/a 210 nm), stored under nitrogen at 4°C.

[0599] Next, the tert-butyloxycarbonyl protecting group on OG1785 was removed using trifluoroacetic acid (TFA) to produce OG1786 as depicted in FIG. 2I.

Example 7 - Synthesis of Polymer OG1801

[0600] Polymer OG1801 is made first from the initiator OG1786. OG1801 has an amine functionality, which is more stable (than maleimide) during polymer synthesis. To synthesize polymer OG1801, a modified version of ATRP is used wherein the copper species (Cu(I)) is generated in situ by adding metallic copper to Cu (II). Starting materials and reagents needed in the reaction are calculated based on batch input of the monomer (HEMA-PC) OG47, as well as the targeted molecular weight (MW).

[0601] Weighed 50 g monomer OG47 in glove box and added 200 mL of degassed EtOH to dissolve the monomer at room temperature; sampled for monomer concentration test. Weighed Cu (II), Bpy, Cu(0) in a 500 mL flask; purged with Argon, while adding monomer solution to the flask; sealed the flask with stopper and vacuumed for 25 min until no bubbles. The reaction changed color gradually from light green to dark green, then to light brown; weighed ~200 mg of initiator OG1786 in glove box, and dissolved in ~2000 uL of DMF under room temperature to make 100 mg/mL stock solution; sampled for initiator concentration and purity test; added the

initiator solution to the flask under Argon. The reaction solution became dark brown and started thickening over time; sealed the system and let the reaction occur over 2 days.

[0602] OG1801 was then prepared for addition of the maleimide and catalyst (copper) was removed as follows: A prepacked RediSep® Rf normal phase silica column is used to remove the catalyst. The size of the column is chosen based on the copper amount in the reaction mixture. For instance, a 330 g column (Cat. # 69-2203-330, Column size 330g, CV=443 mL) was used for a 50 g batch of OG1801. Teflon tubing is used for all the connection as EtOH is the elute solvent.

[0603] After copper removal, all the fractions were transferred to a round bottom flask in batches, and evaporated the EtOH by rotary evaporator at 45-50 °C at reduced pressure to dryness. In this step, EtOH volume collected from condensation was monitored to make sure EtOH removal was > 90%. The polymer was dissolved in 250 mL of WFI and filtered using a 0.2 um filter. It resulted in a clear to light yellow polymer solution at ~150 mg/mL. The solution could be stored at 2-8 °C up to 3 month before use.

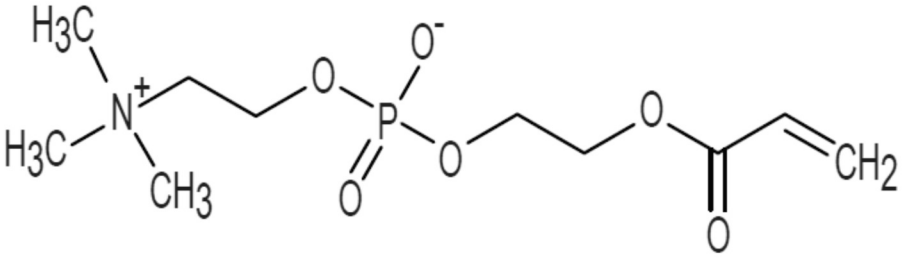
Example 8 - Synthesis of Polymer OG1802

[0604] Starting materials and reagents needed in the reaction are calculated based on batch input of OG1801. The linker is 3-maleimidopropionic acid, NHS ester. Added 30 ml of 0.5 M sodium phosphate (in WFI, pH 8) to 50 g polymer solution (~150 mg/mL). Let stir for 1 min; pH was 8.0 by pH paper. Weighed 204.8 mg of linker and dissolved in DMF 4.1 mL to make 50 mg/mL stock sln. Added linker solution dropwise 815 uL per minute to the polymer sln with strong stirring. Took 5 min to added 4095 uL of linker solution. Reacted at room temperature for 30 min. Quenched reaction with 20 mL of 5% acetic acid to achieve a final pH of 5. Filtered the solution using 1L vacuum filter (0.2 um).

[0605] OG1802 (shown in FIG. 2K) is then purified as follows: Milipore cross flow cassettes are used for polymer purification in aqueous system. Started with concentrating the polymer solution to 250 mL (~ 200 mg/mL). Added the fresh WFI from reservoir, and adjusted the flow rate of the fresh WFI feed to the same as the permeate (~ 2mL/min). The UF/DF was set up at 2-8 °C overnight. Typically 2.5 L of WFI was used (10x volume ratio to the polymer solution). A sample of retente was collected for purity test. The targeted purity was > 98%. Filtered the polymer solution by 0.2 μM 1L filter bottle. The polymer solution could be stored at 2-8 °C for up to 3 month before conjugation.

Example 9 - Alternative Phosphorylcholine Polymers

[0606] A HEA-PC polymer was synthesized as described below. HEA-PC (2-(acryloyloxy)ethyl-2-(trimethylammonium)ethyl phosphate), which is an acrylate as opposed to the methacrylate HEMA-PC described above, has the following structure:



HEA-PC

[0607] HEA-PC was polymerized to the initiator shown in Example 5 as compound L.

TABLE 9.0

Reactant	Name	Amount	MW
Initiator	Compound L (see above)	1.65 mg	2505.5
Monomer	HEA-PC	0.461 g	281.24
Catalyst	Cu (I) Bromide	1.2 mg	143.45
Ligand	Tris [2-(dimethylamino)ethyl]amine (Me6TREN)	2.73 mg	230.39
Solvent A	N,N-Dimethylformamide (DMF)	21.85 µl	73.09
Solvent B	Water	0.7 ml	18.02
Solvent C	Methanol	0.7 ml	32.04

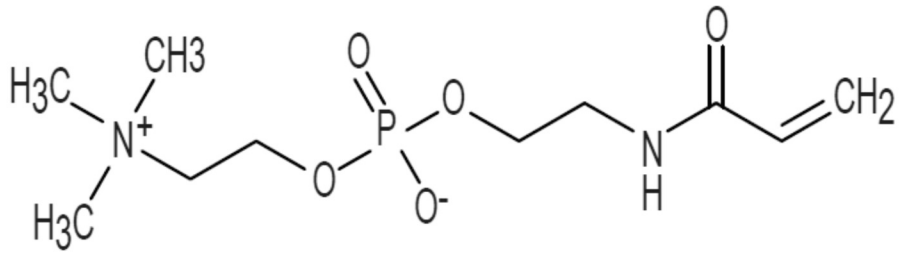
[0608] Prepared a stock solution of initiator at 200mg/mL by dissolving 2.2 mg of initiator in 11 µl of dry DMF and a 200mg/ml solution of ligand by dissolving 4.6 mg of Me6TREN in 23 µL of dry DMF. Dispense 8.25 µl of the stock solution of initiator and 13.6 µl of the ligand into a tube. Degas at -78°C for 5 mn then refill with Argon and add 1.2 mg of CuBr. Degas and

refill with Argon. Add a stock solution of HEA-PC in methanol (weigh out 0.461 g of HEA-PC and dissolve it in 0.5 mL of methanol) to the solution inside the reactor at -78°C. Rinse the vial with 200 µl of methanol and add it inside the reactor at -78°C and then 0.5 mL of distilled water then another 200 µl of water. Degas thoroughly until no bubbling is seen and all heterogeneity disappears (solid particulates dissolve or disappear). Refill with 4 psi of Argon and let the reaction to proceed at RT for an hour. The reaction was already viscous. The reaction was allowed to proceed for about one hour. A solution of bipyrindine in methanol (5mg in 0.5uL) was added. Another 2-3 ml of methanol was added and the catalyst was allowed to oxidize overnight at 4°C. Conversion determined by 1H NMR was estimated to be 94%.

[0609] The next day the polymer was dialyzed and subjected to SEC/MALS analysis using Shodex SB806M_HQ column (7.8x300mm) in 1x PBS pH 7.4 at 1ml/min, giving a PDI of 1.157, Mn of 723.5 kDa, Mp of 820.4 kDa and Mw of 837.2 kDa (before dialysis PDI is 1.12, Mn = 695 kDa, Mp = 778 kDa). Next a maleimide functionality was added to the polymer so that it could be conjugate to a protein.

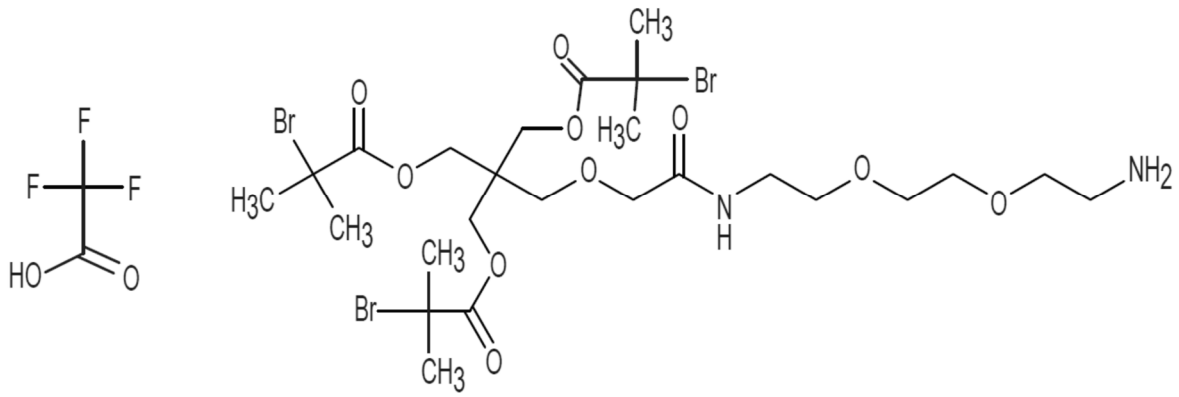
[0610] Next, the maleimide Mal-PEG4-PFP (see Example 20 above) ester was snapped on to the HEA-PC polymer as shown in Example 20. The resulting maleimide functionalized HEA-PC polymer can then be conjugated to sulfhydryl groups as discussed herein for HEMA-PC polymers.

[0611] An acrylamide PC polymer was also made using the monomer 2-(acrylamyl)ethyl-2-(trimethylammonium)ethyl phosphate (Am-PC), having the following structure:



Am-PC

[0612] The Am-PC was used for polymerization employing a 3 arm initiator (a TFA salt) having the structure:



[0613] The synthesis of the Am-PC polymer was conducted as follows:

TABLE 9.1

Reactant	Name/Identity	Amount	MW
Initiator	3-arm initiator (see above)	2.2 mg	885.35
Monomer	Am-PC	0.5 g	280.26
Catalyst (I)	Copper (I) Bromide	1 mg	143.45
Catalyst (II)	Copper (II) Bromide	0.2 mg	223.35
Ligand	Tris[2-(dimethylamino)ethyl]amine (Me6TREN)	3.94 mg	230.39
Solvent A	N,N-Dimethylformamide (DMF)	31.7 µl	73.09
Solvent B	Water	1 ml	18.02
Solvent C	Methanol	1 ml	32.04

[0614] A stock solution of ligand at 200mg/mL was prepared by dissolving 9 mg of Me6TREN in 45uL of dry DMF. Add 19.7uL of the stock solution to a reaction vessel. Prepare a stock solution of initiator at 200mg/mL by dissolving 6.5 mg of material in 32.5uL of DMF. Add 11uL of the initiator stock solution to the ligand from above. Degas for 5 min. Add 1 mg of CuBr. Prepared a stock solution of $CuBr_2$ at 200mg/mL by dissolving 4mg $CuBr_2$ in 20 µL of DMF. Add 0.5g of monomer (AmPC) to 1 mL of methanol (slow dissolution/viscous solution), followed by 1uL of the stock solution of $CuBr_2$. Add the monomer solution dropwise to the

reaction mixture above. Rinse with 1 mL of water. Degas the reaction mixture thoroughly (freeze-thaw). Let the reaction proceed for 24 hours.

[0615] Afterwards the Am-PC polymer may be dialyzed. The molecular weight of the above polymer was determined by SEC/MALS: Mn is 215kDa, Mp: 250kDa, PDI is 1.17. Conversion was estimated by 1H NMR to be 94%. A maleimide functionality can be added to the Am-PC polymer as discussed above for HEMA-PC and HEA-PC. Maleimide functionalized Am-PC polymer can be conjugated to a protein as described above.

Example 10 - Reverse Ellman's Assay for Calculating Free Maleimide in a Compound

[0616] After addition of the maleimide functionality to polymer OG1801 to form OG1802 (see above), an Ellman's assay was used to determine the amount of functional maleimide expressed as percent function (i.e. conjugatable) in a sample. Thiol converted Ellman's reagent (DTNB) to TNB- then to TNB2- in water at neutral and alkaline pH, which gave off a yellow color (measured at 412nm). A standard curve was established with cysteine. Since the maleimide reacts with thiol, this assay actually measured the thiol (cysteine) left. The inhibition was calculated as the molarity ratios of (original thiol – thiol left after maleimide polymer addition)/ (original thiol) and is expressed as a percentage where the higher the percent the higher the maleimide functionalization.

[0617] Reagents Employed in Assay: A standard curve was prepared using the cysteine from 62.5µM to 2µM. Polymer stock solutions were prepared by dissolving the powder in 1xPBS pH7.4 (reaction buffer) and mixing thoroughly. An equal molar of polymer and cysteine solutions were mixed and allowed to react at 27°C for 30 minutes. The 150µM of DTNB solution was added into the cysteine standards and polymer/cysteine reactions and the color was developed at 27°C for 5 minutes. OD at 412nm was read on the Spectramax plate reader and percent inhibition was calculated with the Softmax Pro software and the cysteine standard curve.

Example 11 - Purification and Decapping of antiIL-6-VEGF trap molecules

[0618] The heavy and light chains may be cloned into expression plasmids and transfected into CHO cells. Cells can be grown up in appropriate media and harvested. The antibody (or in the alternative the Ab-Trap) may be purified using Protein A affinity column capture and elution. The antibody (or in the alternative the Ab-Trap) cysteine at position 443

(L443C (EU numbering)) residue is typically "capped" or oxidized by chemicals in the cell culture media and is not available for conjugation. In this regard, purified antibody (or in the alternative the Ab-Trap) may be subjected to a decapping (i.e. reducing) procedure to remove the cap and enable the free (i.e. those not involved in Cys-Cys disulfide bonds) cysteine residue to be conjugated to the maleimide functionality of a polymer. Decapping may be done by mixing purified antibody (or in the alternative the Ab-Trap) protein with a 30x molar excess for 1 hour at ambient temperature of a reducing agent such as TCEP (3,3′,3″-Phosphanetriyltripropanoic acid), Trisodium 3,3',3"-phosphinetriyltris(benzene-1-sulphonate) (TPPTS), or something similar. The reduction reaction may be monitored by SDS-PAGE. Following reduction, the antibody (or in the alternative the Ab-Trap) protein may be buffer exchanged using a Pellicon XL Ultrafiltration Cassette with 20mM Tris pH7.5, 100mM NaCl, 0.5mM TCEP buffer to remove the cap. The TCEP reagent may then be removed in the same buffer exchange setup with 20mM Tris pH7.5, 100mM NaCl. Reduced antibody (or in the alternative the Ab-Trap) may then be allowed to reoxidized using 15x molar excess of the oxidation agent DHAA (DeHydroxy Ascorbic Acid) for 1 hr at ambient temperature which again is monitored by SDS-PAGE assay. The DHAA reagent may then be removed in the same buffer exchange setup with 20mM Tris pH7.5, 100mM NaCl.

Example 12A - Conjugation of antibody (or in the alternative the Ab-Trap) to MPC Polymer

[0619] Decapped antibody (or in the alternative the Ab-Trap) may be conjugated to polymer OG1802 to yield the bioconjugate. An excess of OG1802 is used (3-20 fold molar excess). Conjugation can be monitored by cation-exchanger HPLC chromatography and driven to near completion. Antibody (or in the alternative the Ab-Trap) conjugate may be purified via cation exchanger chromatography and buffer exchanged into the formulation buffer by ultrafiltration/diafiltration (UF/DF). Antibody (or in the alternative the Ab-Trap) conjugate may be purified chromatographically as described above.

Example 12B--VEGFR-AntiIL6 conjugation reaction with OG1802

[0620] VEGFR-AntiIL6 conjugation process with OG1802 involved two steps: an initial reduction reaction to remove the cysteine S protecting groups (decapping), which was followed by conjugation to the biopolymer OG1802 via maleimide-cysteine chemistry. The

decapping was done by first reducing (30-60 min) VEGFR-AntiIL6 with 30x molar excess of 3,3′,3″-Phosphanetriyltripropanoic acid (TCEP) followed by buffer exchange into 20 mM Tris-HCl pH 7.5, 100 mM NaCl to remove the cysteine cap and TCEP (FIG. 28). In the alternative, other reducing agents such as 3′,3″-Phosphanetriyltris(benzenesulfonic acid) trisodium salt (TPPTS) could also be used for the decapping step.

[0621] Then, reduced VEGFR-AntiIL6 was oxidized (30-60 min) with 15x molar excess of dehydro-ascorbic acid (dHAA) followed by buffer exchange into 20 mM Tris pH 7.5, 100 mM NaCl to remove the oxidizing reagent (FIG. 28). Cumulative yield for the decapping procedure was 89% (TCEP reduction – 94%, dHAA oxidation – 95%).

[0622] The conjugation process was done by mixing VEGFR-AntiIL6 at a final concentration of 2.4 mg/mL (or 12.5 μM) with 5x molar excess of the biopolymer OG1802 at pH 8.0. The conjugation process took place at 2-8 °C for a period of 2 to 3 days. Conjugated VEGFR-Anti-IL6 was purified from unconjugated material by cation exchange chromatography (CEX) with Poros XS resin using a step gradient of increasing salt concentrations (0-500 mM NaCl) in buffer containing 20 mM sodium acetate pH 5.5 (FIG. 29). The fractions containing the bioconjugate VEGFR-AntiIL6-OG1802 were pooled and buffer exchanged into phosphate-buffered saline (PBS) pH 7.4 buffer. The conjugation efficiency was approximately 60% (determined by non-reducing SDS-PAGE and ImageJ analysis). The cumulative yield from initial protein material to bioconjugate was approximately 27%.

[0623] SDS-PAGE (FIG. 30) and SEC-MALS (FIG. 31) analysis demonstrate one VEGFR-AntiIL6 molecule (~189 kDa) conjugated with one OG1802 polymer molecule (~794 kDa), which resulted in a bioconjugate of approximately ~983 kDa. SEC-MALS experiment was carried on by injecting 10 μg (protein mass) at flow rate 1 mL/min into a size exclusion chromatography column (Shodex-SB806M-HQ) pre-equilibrated with PBS buffer, in line with a light scattering (miniDAWN TREOS) and refractive index (Optilab T-rEX) detectors (Wyatt Technology). Data and protein conjugate analysis was done in ASTRA 7 (Wyatt Technology).

Example 13 – VEGF Trap Cleavage Identification and Removal

[0624] As shown in FIGs. 11A and 11B, SDS-PAGE of protein A purified VEGFR-Anti-IL-6 and Anti-IL-6-VEGFR reduced products derived from ExpiCHO-S cells displayed unexpected bands with sizes dependent on the construct orientations. These bands indicated that a fraction of the dual inhibitor molecules underwent cleavage during production. In this example, VEGFR-AntiIL6 corresponds to a heavy chain molecule comprising of sequences 1A-2A-3H paired with a light chain containing sequence 4A. AntiIL-6 VEGFR corresponds to a heavy chain comprising of sequences 5H(Fab)-2A-1A-5H(Fc) paired with a light chain containing sequence 4A.

[0625] As illustrated in FIG. 12, these protein bands were transferred to a PVDF membrane and the first six residues of the protein bands were sequenced through Edman's N-terminal sequencing (Procise 494HT instrument). Results showed that cleaved products share the same N-terminal sequence (93LTHRQT), which is located within the VEGFR1 domain 2 at the VEGF trap region.

[0626] FIGs. 13A and 13B show the results of the mutations at the region flanking the cleavage site (K89TNY/LTHR96) that would disrupt protein cleave while maintaining the protein overall stability and binding to VEGF properties. Those mutations were chosen based on sequence conservation, for this purpose the Basic Local Alignment Search Tool (BLAST) was used to do a sequence similarity search and alignment between VEGFR1 domain 2 sequence and protein homologs within the Mammalia class. The 500 best hits were selected based on their BLAST E value score. From this initial cluster containing 500 sequences (cluster_1), the best scoring sequence for each individual species was selected reducing it to a set of 106 representative sequences (cluster_2). Based on sequence variations within the cleavage site flanking region observed in cluster_2, 16 Anti-IL-6-VEGFR single point mutants were generated and evaluated on the protein cleavage. These mutations were initially only introduced in the Anti-IL-6-VEGFR construct since it shows a better size separation between cleaved and intact products due to their molecular weight differences (~35 kDa).

[0627] FIGs. 13A and 13B illustrate SDS-PAGE results of Anti-IL-6 VEGFR mutants demonstrating that modifications at the cleavage site tended to reduce the amount of cleaved product (indicated by arrows). Mutations T94I and H95I (residue numbering based on position at the VEGF trap sequence) markedly reduced the VEGF trap cleavage. Specifically, FIGs. 13A and

13B illustrate that most of the mutations reduced protein cleavage and mutations T94I (VEGFR_variant_1), illustrated in FIG. 13C, and H95I (VEGFR_variant_2), illustrated in FIG. 13D, had a more marked effect. Single point mutation (T94I, H95I) and double point mutation (T94I/H95I, VEGFR_variant_3), as illustrated by FIG. 13E, were subsequently introduced in the VEGFR-Anti-IL-6 construct. FIG. 13C indicates the position of T94I (underlined). FIG. 13D indicates the position of H95I (underlined). FIG. 13E indicates the position of T94I and H95I (underlined). As illustrated in FIGs. 13F and 13G, SDS-PAGE showed that similar reduction in protein cleavage was observed and this effect is further enhanced when double mutation is introduced.

Example 14 – Dual Inhibitor VEGFR Variants Binding to VEGF Via Biacore

[0628] FIGs. 14A-14F illustrate the results for a series of Biacore assays used to determine the affinity between Anti-VEGF agents and VEGF. Binding kinetics between molecules were measured using a Biacore T200 (GE Healthcare) at 37 °C in buffer HBS-EP+ containing 1 mg/mL BSA. Anti-VEGF agents (VEGFR-Anti-IL-6 (5 µg/mL), VEGFR_variant_1-Anti-IL-6 (5 µg/mL), VEGFR_variant_2-Anti-IL-6 (5 µg/mL), VEGFR_variant_3-Anti-IL-6 (5 µg/mL) and Eylea (3 µg/mL)) were captured on a Protein A chip (GE) at 10 µL/min for 25 seconds, five concentrations of VEGF-A165 (0.19, 0.56, 1.67, 5, 15 nM) were flowed over captured antibodies for 120 seconds at 30 µL/min and dissociated for 60 min. The sensor chip surface was regenerated by 60 seconds injection of 10 mM Glycine, pH 1.7 at a flow rate of 50 µl/min. All sensorgrams were double reference subtracted and fit using a 1:1 Langmuir binding model. FIGs. 14A-14F show the introduction of single point mutations T94I (VEGFR_variant_1-Anti-IL-6), H95I (VEGFR_variant_2-Anti-IL-6) and double mutant T94I/H95I (VEGFR_variant_3-Anti-IL-6) minimally affects binding to VEGF. As can be seen in FIG. 14F, sensograms demonstrate dual inhibitor molecules VEGFR-Anti-IL-6 and variants bind tightly to VEGF-A as Eylea. In this example, the VEGFR-AntiIL6 molecules correspond to VEGFR variants (Sequences 1A-1D) fused to the AntiIL-6 moiety via the GS linker (sequences 2A-3H) paired with light chain containing sequence 4A.

Example 15 – Cell Based VEGF Stimulated VEGFR Reporter Assay

[0629] The results of a cell-based VEGF stimulated VEGFR reporter assay are provided in FIGs. 15. Dual inhibitorVEGFR-AntiIL6, as noted above, blocks VEGF from binding VEGFR and stimulating downstream signaling to the same degree as Eylea (IC50 0.18nM vs. 0.11nM), while the anti-IL-6 antibody did not have any effect.

[0630] For the experiments, Promega KDR/VEGF HEK293 thaw-and-use cells (CS181405; Promega) were thawed and added to 4.6 mL of assay medium (10%FBS in DMEM). 25 µL of cells were transferred to each well of a 96-well plate. VEGF (500 pM final concentration) was mixed with a 10-point, 1:3 dilution series of inhibitors, starting at 100 nM (final concentration), and incubated for 30 minutes before adding 50 µL to the plated cells. After 6 hours, 75 µL of prepared Bio-Glo reagent (Promega) was added to the cells and incubated for 3-10 minutes at room temperature. Luminescence was measured on an iD3 plate reader (Molecular Devices). Relative luminescence units were normalized to the lowest inhibitor concentration for each curve and plotted as the average of replicates with standard error against the log of the concentration of antibody using GraphPad Prism software and fit with a non-linear regression curve. In this example, VEGFR-AntiIL6 corresponds to a heavy chain molecule comprising of sequences 1A-2A-3H paired with a light chain containing sequence 4A.

Example 16 – HUVEC Tubule Formation Assay

[0631] FIGs. 16A-16C illustrate the results of HUVEC tubule formulation assay.

[0632] Passage 2 or 3 HUVEC cells were cultured in Vascular Basal Media supplemented with 1x Bovine Brain Extract endothelial cell growth kit (ATCC; growth media) in 5% CO2 at 37 °C. Once cells reached 70% confluency, cells were lifted from the plate using trypsin (0.05%; ATCC) and 0.75x10^6 cells plated in a T25 flask in growth media. Media was swapped for Assay Media (2% FBS in Vascular Basal Medium) the following day. After 6 hours, 5 µg/ml of Calcein AM was directly added to the media and incubatet for 30 minutes at 37 °C. At the same time, 200 µL of pre-thawed Matrigel (growth factor reduced; Corning) were added to each well of an 8-well glass chamber slide and incubate at 37 °C for 30 minutes. During incubations, 25 nM of each inhibitor (final concentrations) was mixed with 3 nM VEGF (R&D), 12.5 nM IL6 (Tonbo), and 25 nM IL6R (Tonbo) (all final concentrations). Following the 30 minute Calcein AM incubation, the cells were washed 3x with PBS and then trypsinized. Harvested cells

were centrifuged for 3 minutes at 1,500xg and resuspended in assay medium at a density of 500,000 cells/mL. 100 µL of cells were then mixed with 100 µL of prepared inhibitor/complex solutions, and then transferred to the Matrigel coated chamber slides and incubated overnight. The next day, tubules were imaged using a 10x objective lens and the GFP filter on a EVOS fluorescence microscope (Life Technologies). At least 2 representative images were taken for each condition and then analyzed using the Angiogenesis Analyzer Plugin for ImageJ (NIH), which evaluates and quantifies 20 tubule network formation parameters.

[0633] FIGs. 16A-16C show data as average with standard error from 4 independent experiments. Statistical significance was determined from all replicates from 4 independent experiments using an unpaired, two-tailed Students t-test. As illustrated in FIG. 16C, VEGFR-AntiIL6 significantly inhibited angiogenic parameters in 17/20 parameters as compared to the control. In contrast, Eylea inhibited in 4/20 parameters and anti-IL-6 inhibited in 7/20 parameters. VEGFR-AntiIL6 was significantly better than Eylea and Anti-IL-6 in 12/20 parameters, as indicated by asterisks in FIG. 16B. Furthermore, t-test statistics are presented in FIG. 16C. In this example, VEGFR-Anti-IL6 corresponds to a heavy chain molecule comprising of sequences 1A-2A-3H paired with a light chain containing sequence 4A.

[0634] In some embodiments, the VEGFR-Anti-IL6 construct allows for synergistic mechanisms to improve efficacy and durability in specific at-risk populations. At-risk populations include those with at-risk for or diagnosed with diabetic retinopathy or one or more inflammatory retinal disease.

Example 17 – HUVEC Proliferation Assay

[0635] FIG. 17 illustrates the results of an HUVEC proliferation assay. HUVEC cells (passages 2-5) were cultured in Vascular Basal Media supplemented with 1x Bovine Brain Extract endothelial cell growth kit (ATCC; growth media) in 5% CO2 at 37 degrees. Once cells reached 70% confluency, cells were lifted from the plate using trypsin (0.05%; ATCC) and 3,500 cells/well were seeded in the middle 60 wells of a 96-well gelatin coated plate in 100 uL of Assay Media (Vascular Basal Media supplemented with l-glutamine, ascorbic acid, heparan sulfate, hydrocortisone, and 1%FBS) (Corning). After 24 hours of starvation, cells were stimulated with VEGF (1.5 nM), IL-6 (5 nM), and IL6R (10 nM) mixed with 10-point, 1:3 dilution series of

inhibitors starting at 100 nM. These concentrations of VEGF and IL-6 induce proliferation at sub-maximal levels when added independently of each other, but demonstrated a synergistic growth curve when combined. 48 hours later, cell viability was measured using CellTiterBlue reagent (Promega) according to manufacturer's instructions on an iD3 plate reader (Molecular Devices). For absorbance measurements, the ratio of A570/A605 was determined and data were normalized to control (no inhibitor) cells for each replicate. Fluorescence measurements (555ex/605em) were normalized to control cells for each replicate.

[0636] As shown in FIG. 17, normalized values were plotted as the average of replicates with standard error against the concentration of antibody using GraphPad Prism software and fit with a non-linear regression curve.

[0637] As can be seen in FIG. 17, VEGFR-AntiIL6 dual inhibitor blocked synergistic stimulation of HUVEC proliferation by IL-6 and VEGF, while neither Eylea nor anti-Il-6Anti-IL-6 affected HUVEC growth under these conditions.

Example 18– Generation of Improved Anti-IL-6 Paratopes

[0638] The generation of improved Anti-IL-6 paratopes is described in the present example. Biacore kinetics results are shown in Tables 18.1, 18.2, 18.3, and 18.4

[0639] To provide a superior anti-IL-6 paratope on the engineered human IgG1 framework, the G66D (CDR2) heavy chain mutant was affinity matured. Initially, G66D full heavy chain libraries containing random single point mutations at positions S35, I51, T63 and T65 were constructed, excluding substitutions for Cys, Met and Asn due to the chemical liabilities associated with these amino acids. Those four positions were selected based on their tolerance for mutations as observed in bacterial and mammalian screens. The mammalian expression vectors containing heavy chain mutations were co-transfected with light chain wild-type and variant M32L/M50D/N52S/M88Q into 3 mL cultures of Expi293 cells and incubated for five days. These cultures were centrifuged and supernatants containing secreted antibodies were harvested and diluted in HBS-EP+ running buffer (1:20 ratio).

[0640] The various constructs and resulting kinetic properties are provided below in Tables 18.1-18.4.

Table 18.1

Biacore Kinetics for Anti-IL-6 Heavy Chain Double Mutants

		Sup / 37 °C						Purified / 37 °C					
Heavy chain - mutatio	Light chain - mutation	Capture (RU)	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)	Capture (RU)	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
WT	WT	561.6	6.70E+05	2.55E-04	3.81E-10	125.50	0.0342	82.5	5.65E+05	1.28E-04	2.26E-10	17.4	0.171
G66D	WT	878.9	1.83E+06	3.92E-04	2.15E-10	198.70	0.189	90.5	1.57E+06	1.78E-04	1.13E-10	20.4	0.166
S35H , G66D	WT	1084.6	2.92E+06	3.97E-04	1.36E-10	231.60	0.839	78.1	1.77E+06	1.85E-04	1.05E-10	17.1	0.169
S35G , G66D	WT	167	1.27E+06	3.60E-04	2.84E-10	39.60	0.0144						
S35R , G66D	WT	3.6	1.22E+02	2.95E-04	2.42E-06	0.00	0.0346						
S35Y , G66D	WT	129.2	1.10E+06	5.51E-04	5.03E-10	30.60	0.043						
S35P , G66D	WT	48.4	1.03E+05	1.23E-03	1.19E-08	8.30	0.016						
S35W , G66D	WT	161.5	9.23E+04	2.17E-03	2.35E-08	33.10	0.0784						
S35I , G66D	WT	384.4	1.12E+06	3.76E-04	3.34E-10	87.40	0.0524						
S35V , G66D	WT	856.3	1.13E+06	4.69E-04	4.14E-10	181.50	0.0742						
S35A , G66D	WT	723.8	1.65E+06	4.94E-04	3.00E-10	168.80	0.076						
S35D , G66D	WT	577.1	2.00E+06	6.41E-04	3.21E-10	147.80	0.0814						
S35L , G66D	WT	257.5	1.11E+06	3.51E-04	3.15E-10	57.70	0.0273						
S35T , G66D	WT	674.3	1.38E+06	4.09E-04	2.97E-10	146.20	0.117						
S35K , G66D	WT	16.4	1.70E+06	9.49E-04	5.57E-10	4.50	0.0309						
S35E , G66D	WT	137.7	8.74E+05	5.09E-04	5.82E-10	31.00	0.0558						
S35F , G66D	WT	143.6	1.59E+06	1.19E-03	7.49E-10	35.20	0.0522						
S35Q , G66D	WT	390.6	1.31E+06	3.95E-04	3.02E-10	84.90	0.0382						
[redacted]													
I51R , G66D	WT	139.9	1.20E+06	2.02E-04	1.69E-10	31.60	0.0962						
I51V , G66D	WT	-10.4	1.81E+06	6.68E-06	3.69E-12	0.80	0.32						
I51W , G66D	WT	559.1	1.91E+06	9.43E-03	4.93E-09	120.50	1.38						
I51Y , G66D	WT	433	1.48E+06	5.10E-03	3.45E-09	100.60	0.449						
I51S , G66D	WT	402.8	1.89E+06	3.62E-04	1.91E-10	94.90	0.186						
I51A , G66D	WT	668.5	1.76E+06	5.76E-04	3.27E-10	149.70	0.203						
I51E , G66D	WT	57.2	7.54E+05	7.60E-04	1.01E-09	10.70	0.056						
I51T , G66D	WT	435.2	1.79E+06	4.15E-04	2.32E-10	102.10	0.107						
I51D , G66D	WT	71.4	9.91E+05	2.67E-03	2.69E-09	12.40	0.0255						
I51F , G66D	WT	367.9	1.36E+06	3.05E-03	2.24E-09	82.90	0.13						
I51L , G66D	WT	519.6	1.90E+06	7.33E-04	3.86E-10	118.50	0.1						
I51K , G66D	WT	169.6	1.38E+06	4.02E-04	2.91E-10	40.60	0.0621						
I51P , G66D	WT	34	1.58E+04	6.12E-03	3.87E-07	9.80	0.0467						
I51H , G66D	WT	249	1.52E+06	5.29E-04	3.48E-10	57.70	0.075						
I51Q , G66D	WT	262	1.93E+06	6.48E-04	3.36E-10	61.40	0.1						
I51G , G66D	WT	608	1.36E+06	5.81E-04	4.28E-10	135.50	0.0597						
[redacted]													
T63S , G66D	WT	723.7	2.52E+06	4.64E-04	1.84E-10	163.30	0.367						
T63I , G66D	WT	682.7	1.77E+06	3.73E-04	2.11E-10	153.20	0.156						
T63H , G66D	WT	811.6	1.98E+06	4.20E-04	2.13E-10	179.40	0.287						
T63E , G66D	WT	630.3	2.61E+06	4.64E-04	1.78E-10	144.80	0.289	77.9	1.72E+06	2.52E-04	1.47E-10	17.1	0.151
T63V , G66D	WT	708.8	1.84E+06	3.99E-04	2.17E-10	158.80	0.249						
T63G , G66D	WT	585.7	1.86E+06	3.97E-04	2.13E-10	131.30	0.166						
T63K , G66D	WT	531.3	2.49E+06	4.61E-04	1.85E-10	120.50	0.333	77	1.46E+06	2.35E-04	1.61E-10	16.9	0.173
T63W , G66D	WT	615.9	1.70E+06	4.27E-04	2.51E-10	136.30	0.143						
T63Q , G66D	WT	673.7	2.01E+06	4.08E-04	2.03E-10	150.00	0.255	70.9	1.30E+06	2.51E-04	1.93E-10	16.2	0.204
T63R , G66D	WT	729	2.66E+06	4.60E-04	1.73E-10	165.20	0.384						
T63L , G66D	WT	698.9	1.73E+06	3.63E-04	2.10E-10	158.80	0.167						
T63D , G66D	WT	684.8	1.92E+06	3.92E-04	2.04E-10	154.80	0.103						
T63A , G66D	WT	632.4	2.80E+06	4.96E-04	1.77E-10	142.50	0.361						
T63Y , G66D	WT	701.2	1.82E+06	3.83E-04	2.10E-10	157.00	0.169						
T63F , G66D	WT	621.5	1.81E+06	4.01E-04	2.21E-10	137.80	0.169						
T63P , G66D	WT	481.3	2.08E+06	4.36E-04	2.10E-10	110.50	0.203						
[redacted]													
T65K , G66D	WT	909.7	1.57E+06	4.12E-04	2.62E-10	199.50	0.226						
T65D , G66D	WT	648.1	2.18E+06	4.42E-04	2.03E-10	146.60	0.378						
T65L , G66D	WT	662.8	1.77E+06	3.75E-04	2.12E-10	148.80	0.363						
T65W , G66D	WT	749	1.64E+06	3.70E-04	2.26E-10	154.50	0.508						
T65Q , G66D	WT	863.8	3.28E+06	5.17E-04	1.58E-10	192.10	1.68	87.4	1.77E+06	2.62E-04	1.48E-10	19.1	0.135
T65E , G66D	WT	937.2	2.20E+06	4.27E-04	1.94E-10	207.60	0.358						
T65V , G66D	WT	872.5	2.88E+06	5.04E-04	1.75E-10	193.90	0.555						
T65S , G66D	WT	935.4	1.82E+06	3.83E-04	2.10E-10	206.20	0.244						
T65Y , G66D	WT	593.7	1.61E+06	3.61E-04	2.24E-10	128.00	0.0985						
T65P , G66D	WT	341.4	1.54E+06	5.68E-04	3.69E-10	80.80	0.0563						
T65A , G66D	WT	690.4	1.64E+06	3.64E-04	2.22E-10	153.40	0.127						
T65H , G66D	WT	659.8	1.63E+06	3.56E-04	2.19E-10	146.80	0.122						
T65R , G66D	WT	730.4	2.00E+06	4.44E-04	2.22E-10	163.50	0.192						
T65G , G66D	WT	56.1	1.77E+06	2.18E-04	1.23E-10	14.20	0.033						
T65F , G66D	WT	587.6	1.56E+06	3.48E-04	2.23E-10	129.10	0.0947						

Table 18.2

Biacore Kinetics for Anti-IL-6 Heavy Chain Double Mutants with Mutagenized Light Chain

			Sup / 37 °C					Purified / 37 °C					
Heavy chain - mutatio	Light chain - mutation	Capture (RU)	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)	Capture (RU)	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)
WT	M32L, M50D, N52S, M88Q	627.9	6.77E+05	4.14E-04	6.11E-10	133.3	0.0463	100.8	1.12E+06	3.19E-04	2.86E-10	19.5	0.133
G66D	M32L, M50D, N52S, M88Q	504.9	1.63E+06	4.83E-04	2.96E-10	110.5	0.0715	72.2	1.69E+06	4.12E-04	2.44E-10	16.2	0.137
S35H , G66D	M32L, M50D, N52S, M88Q	1338.7	3.24E+06	5.39E-04	1.66E-10	270.1	1.6	83.4	1.20E+06	2.54E-04	2.12E-10	17.6	0.151
S35G , G66D	M32L, M50D, N52S, M88Q	76.8	1.28E+06	7.81E-04	6.09E-10	17.1	0.0227						
S35R , G66D	M32L, M50D, N52S, M88Q	11.3	3.79E+03	1.21E-03	3.20E-07	0	0.0397						
S35Y , G66D	M32L, M50D, N52S, M88Q	75.4	1.18E+06	4.04E-03	3.43E-09	16.6	0.121						
S35P , G66D	M32L, M50D, N52S, M88Q	89.3	2.02E+05	5.48E-03	2.71E-08	13.4	0.0853						
S35W , G66D	M32L, M50D, N52S, M88Q	384.2	1.71E+05	5.91E-03	3.46E-08	75.4	0.336						
S35I , G66D	M32L, M50D, N52S, M88Q	519.3	9.04E+05	1.47E-03	1.62E-09	108.3	0.221						
S35V , G66D	M32L, M50D, N52S, M88Q	950.9	1.03E+06	1.37E-03	1.33E-09	194.4	0.0828						
S35A , G66D	M32L, M50D, N52S, M88Q	326.2	1.93E+06	1.26E-03	6.57E-10	73.3	0.107						
S35D , G66D	M32L, M50D, N52S, M88Q	251.8	2.35E+06	1.77E-03	7.53E-10	62.7	0.116						
S35L , G66D	M32L, M50D, N52S, M88Q	461.7	9.42E+05	2.43E-03	2.59E-09	100.2	0.0915						
S35T , G66D	M32L, M50D, N52S, M88Q	265.5	1.43E+06	7.11E-04	4.98E-10	57.2	0.236						
S35K , G66D	M32L, M50D, N52S, M88Q	-1.5	1.31E+06	3.46E-03	2.64E-09	1.80	0.0621						
S35E , G66D	M32L, M50D, N52S, M88Q	14.2	2.18E+06	1.89E-03	8.67E-10	4.90	0.0255						
S35F , G66D	M32L, M50D, N52S, M88Q	38.9	1.53E+06	4.84E-03	3.17E-09	8.10	0.0776						
S35Q , G66D	M32L, M50D, N52S, M88Q	154.9	2.09E+06	6.26E-04	3.00E-10	35.30	0.104						
[redacted]													
I51R , G66D	M32L, M50D, N52S, M88Q	34.4	1.63E+06	5.80E-05	3.57E-11	7.7	0.0516						
I51V , G66D	M32L, M50D, N52S, M88Q	72.6	1.76E+06	1.95E-03	1.11E-09	17.1	0.214						
I51W , G66D	M32L, M50D, N52S, M88Q	46.5	7.91E+05	3.83E-03	4.84E-09	8.2	0.134						
I51Y , G66D	M32L, M50D, N52S, M88Q	48.2	6.10E+06	2.47E-02	4.04E-09	8.9	0.201						
I51S , G66D	M32L, M50D, N52S, M88Q	118.4	1.85E+06	4.01E-04	2.17E-10	28.1	0.136						
I51A , G66D	M32L, M50D, N52S, M88Q	196.7	1.61E+06	8.99E-04	5.59E-10	44.8	0.0388						
I51E , G66D	M32L, M50D, N52S, M88Q	21.2	9.14E+05	1.96E-03	2.14E-09	3.5	0.129						
I51T , G66D	M32L, M50D, N52S, M88Q	84.7	2.05E+06	4.78E-04	2.33E-10	20.4	0.131						
I51D , G66D	M32L, M50D, N52S, M88Q	13.5	8.34E+05	7.99E-03	9.58E-09	2.9	0.0922						
I51F , G66D	M32L, M50D, N52S, M88Q	32.3	1.37E+06	6.11E-03	4.45E-09	6.2	0.238						
I51L , G66D	M32L, M50D, N52S, M88Q	69.4	1.64E+06	1.61E-03	9.80E-10	1.67E+01	0.053						
I51K , G66D	M32L, M50D, N52S, M88Q	42.6	1.72E+06	6.01E-04	3.49E-10	9.00E+00	0.086						
I51P , G66D	M32L, M50D, N52S, M88Q	-4.4	1.25E+06	1.00E-02	8.04E-09	1.00E-01	0.154						
I51H , G66D	M32L, M50D, N52S, M88Q	17.3	2.07E+06	1.04E-03	5.00E-10	5.60E+00	0.0639						
I51Q , G66D	M32L, M50D, N52S, M88Q	43.5	1.96E+06	1.13E-03	5.75E-10	9.40E+00	0.123						
I51G , G66D	M32L, M50D, N52S, M88Q	152	1.40E+06	9.12E-04	6.52E-10	3.63E+01	0.208						
[redacted]													
T63S , G66D	M32L, M50D, N52S, M88Q	292.2	1.80E+06	4.99E-04	2.78E-10	66.8	0.298						
T63I , G66D	M32L, M50D, N52S, M88Q	287	1.55E+06	4.46E-04	2.88E-10	64.4	0.0411						
T63H , G66D	M32L, M50D, N52S, M88Q	422.7	1.72E+06	4.81E-04	2.80E-10	93.5	0.138						
T63E , G66D	M32L, M50D, N52S, M88Q	325.9	2.00E+06	4.85E-04	2.42E-10	75.2	0.13	63.5	1.65E+06	3.27E-04	1.98E-10	14.2	0.154
T63V , G66D	M32L, M50D, N52S, M88Q	263.7	1.57E+06	4.30E-04	2.74E-10	59	0.0516						
T63G , G66D	M32L, M50D, N52S, M88Q	136.6	1.73E+06	4.50E-04	2.61E-10	29.8	0.11						
T63K , G66D	M32L, M50D, N52S, M88Q	626.9	2.15E+06	5.29E-04	2.46E-10	140.5	0.233	63.5	1.59E+06	4.01E-04	2.52E-10	14.4	0.165
T63W , G66D	M32L, M50D, N52S, M88Q	153.7	1.57E+06	4.49E-04	2.86E-10	34.1	0.051						
T63Q , G66D	M32L, M50D, N52S, M88Q	435.4	1.78E+06	4.61E-04	2.59E-10	96.6	0.164	61.9	1.69E+06	3.41E-04	2.01E-10	14.1	0.108
T63R , G66D	M32L, M50D, N52S, M88Q	391.6	1.92E+06	5.26E-04	2.73E-10	88.7	0.184						
T63L , G66D	M32L, M50D, N52S, M88Q	473.4	1.60E+06	4.54E-04	2.83E-10	105.1	0.0607						
T63D , G66D	M32L, M50D, N52S, M88Q	294.9	1.66E+06	4.13E-04	2.49E-10	6.76E+01	0.0916						
T63A , G66D	M32L, M50D, N52S, M88Q	415.5	1.70E+06	4.58E-04	2.68E-10	9.23E+01	0.155						
T63Y , G66D	M32L, M50D, N52S, M88Q	198.4	1.91E+06	4.59E-04	2.41E-10	4.75E+01	0.266						
T63F , G66D	M32L, M50D, N52S, M88Q	152.7	1.67E+06	4.59E-04	2.74E-10	3.53E+01	0.194						
T63P , G66D	M32L, M50D, N52S, M88Q	144.9	1.70E+06	4.19E-04	2.46E-10	3.14E+01	0.257						
[redacted]													
T65K , G66D	M32L, M50D, N52S, M88Q	1236.8	1.63E+06	5.79E-04	3.56E-10	261	0.301						
T65D , G66D	M32L, M50D, N52S, M88Q	391.9	1.03E+06	4.80E-04	4.65E-10	86.4	0.137						
T65L , G66D	M32L, M50D, N52S, M88Q	490.4	1.53E+06	4.46E-04	2.93E-10	109.5	0.0578						
T65W , G66D	M32L, M50D, N52S, M88Q	324.9	1.19E+06	3.84E-04	3.22E-10	67.8	0.0812						
T65Q , G66D	M32L, M50D, N52S, M88Q	631.4	2.18E+06	5.62E-04	2.58E-10	144.7	0.191	80.2	1.82E+06	3.80E-04	2.09E-10	17.1	0.144
T65E , G66D	M32L, M50D, N52S, M88Q	538.6	1.70E+06	4.74E-04	2.79E-10	118.8	0.268						
T65V , G66D	M32L, M50D, N52S, M88Q	537.7	1.96E+06	5.51E-04	2.81E-10	118.6	0.303						
T65S , G66D	M32L, M50D, N52S, M88Q	563.8	1.42E+06	4.41E-04	3.10E-10	123.7	0.216						
T65Y , G66D	M32L, M50D, N52S, M88Q	313.4	1.55E+06	3.67E-04	2.37E-10	16.5	0.54						
T65P , G66D	M32L, M50D, N52S, M88Q	16.3	1.79E+06	1.08E-03	6.01E-10	4.7	0.673						
T65A , G66D	M32L, M50D, N52S, M88Q	677.3	2.27E+06	5.42E-04	2.39E-10	1.51E+02	0.863						
T65H , G66D	M32L, M50D, N52S, M88Q	385.7	1.43E+06	4.23E-04	2.97E-10	8.58E+01	0.0507						
T65R , G66D	M32L, M50D, N52S, M88Q	986.3	1.63E+06	5.32E-04	3.27E-10	2.10E+02	0.321						
T65G , G66D	M32L, M50D, N52S, M88Q	311.9	1.53E+06	4.77E-04	3.11E-10	7.20E+01	0.111						
T65F , G66D	M32L, M50D, N52S, M88Q	300.9	1.34E+06	3.91E-04	2.91E-10	6.65E+01	0.131						

Table 18.3

Biacore Kinetics for Anti-IL-6 Heavy Chain Triple mutants

		Sup / 37 °C						Purified / 37 °C					
Heavy chain - mutation	**Light chain - mutation**	**Capture (RU)**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU**	**Chi² (RU²)**	**Capture (RU)**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU**	**Chi² (RU²)**
WT	WT	292.6	8.61E+05	5.59E-04	6.49E-10	60.7	0.141	71.1	6.15E+05	1.32E-04	2.14E-10	11.6	0.137
G66D	WT	459.6	1.62E+06	4.80E-04	2.96E-10	98.7	0.176						
S35H, G66D	WT	583.7	1.46E+06	5.48E-04	3.74E-10	118.3	0.212						
S35H, G66D, Q99V	WT	233.5	8.98E+05	4.31E-03	4.80E-09	45.6	0.469						
S35H, G66D, Q99L	WT	15.5	1.07E+06	2.62E-04	2.44E-10	3.9	0.101						
S35H, G66D, Q99F	WT	17.9	4.89E+05	1.66E-03	3.39E-09	1.8	0.166						
S35H, G66D, Q99S	WT	462.8	3.96E+06	2.57E-02	6.48E-09	69.4	0.905						
S35H, G66D, Q99E	WT	456.3	4.93E+05	1.93E-03	3.91E-09	91.6	9.97						
S35H, G66D, Q99R	WT	391.4	1.41E+05	1.61E-03	1.14E-08	49.5	0.331						
S35H, G66D, Q99H	WT	237.2	8.02E+05	1.36E-03	1.70E-09	45	0.423						
S35H, G66D, Q99G	WT	862.2	3.43E+06	3.30E-03	9.61E-10	190.1	0.879						
S35H, G66D, Q99I	WT	56.9	8.68E+05	3.35E-03	3.86E-09	5.4	0.417						
S35H, G66D, Q99D	WT	778	1.29E+06	3.02E-03	2.34E-09	128.6	0.815						
S35H, G66D, Q99A	WT	328.3	1.85E+06	1.32E-03	7.17E-10	57	0.36						
S35H, G66D, Q99K	WT	142.5	2.20E+05	3.33E-03	1.51E-08	21.8	0.719						
S35H, G66D, Q99Y	WT	112.3	2.60E+06	2.15E-03	8.25E-10	12.6	0.191						
S35H, G66D, Q99T	WT	845.8	1.69E+06	2.22E-03	1.31E-09	165.8	0.625						
[redacted]													
S35H, G66D, L100I	WT	525.4	9.31E+05	6.44E-04	6.92E-10	94.2	0.134						
S35H, G66D, L100Y	WT	529.9	1.01E+06	6.42E-04	6.38E-10	101.3	0.0737						
S35H, G66D, L100H	WT	523.5	7.23E+05	4.98E-04	6.88E-10	109.5	0.207						
S35H, G66D, L100R	WT	562	9.51E+05	9.57E-04	1.01E-09	101.6	0.518						
S35H, G66D, L100E	WT	438.7	1.13E+06	8.69E-04	7.66E-10	82.4	0.276						
S35H, G66D, L100D	WT	510.6	1.79E+06	7.99E-04	4.47E-10	102.4	0.426						
S35H, G66D, L100A	WT	870.7	4.52E+06	8.33E-04	1.84E-10	185.5	0.757						
S35H, G66D, L100S	WT	970.4	3.28E+06	6.44E-04	1.96E-10	194.3	0.635						
S35H, G66D, L100G	WT	1113.1	3.20E+06	9.60E-04	3.00E-10	228.6	0.655						
S35H, G66D, L100T	WT	820.2	3.34E+06	7.24E-04	2.17E-10	168.7	0.635						
S35H, G66D, L100V	WT	826.9	2.37E+06	5.79E-04	2.44E-10	151.4	0.515						
S35H, G66D, L100Q	WT	903.7	3.56E+06	6.40E-04	1.80E-10	181.4	0.88						
S35H, G66D, L100K	WT	882.8	5.88E+06	1.06E-03	1.80E-10	174.7	3.31						
S35H, G66D, L100F	WT	1002.3	2.51E+06	6.12E-04	2.44E-10	194.6	0.752						

Table 18.4

Biacore Kinetics for Anti-IL-6 Heavy Chain Triple Mutants Paired with Mutagenized Light Chain

		Sup / 37 °C						Purified / 37 °C					
Heavy chain - mutation	**Light chain - mutation**	**Capture (RU)**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU**	**Chi² (RU²)**	**Capture (RU)**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Rmax (RU**	**Chi² (RU²)**
WT	M32L, M50D, N52S, M88Q	239.2	9.20E+05	6.31E-04	6.86E-10	48.3	0.259						
G66D	M32L, M50D, N52S, M88Q	149.1	1.66E+06	6.54E-04	3.93E-10	32.2	0.401						
S35H, G66D	M32L, M50D, N52S, M88Q	644	1.50E+06	6.07E-04	4.03E-10	128.2	0.329	68.7	1.22E+06	2.38E-04	1.95E-10	12	0.172
S35H, G66D, Q99P	M32L, M50D, N52S, M88Q	421.1	1.25E+06	1.69E-03	1.35E-09	81.4	0.893						
S35H, G66D, Q99V	M32L, M50D, N52S, M88Q	9.7	7.94E+05	1.40E-03	1.76E-09	1.3	0.108						
S35H, G66D, Q99L	M32L, M50D, N52S, M88Q	6.1	1.30E+06	7.75E-04	5.95E-10	1	0.19						
S35H, G66D, Q99F	M32L, M50D, N52S, M88Q	56.4	1.14E+06	1.13E-04	9.97E-11	0.5	0.36						
S35H, G66D, Q99S	M32L, M50D, N52S, M88Q	599.7	2.01E+05	1.54E-03	7.66E-09	85.4	3						
S35H, G66D, Q99E	M32L, M50D, N52S, M88Q	609.4	1.89E+05	8.90E-04	4.71E-09	148.2	0.726						
S35H, G66D, Q99W	M32L, M50D, N52S, M88Q	5.6	1.12E+11	3.20E-03	2.85E-14	0.4	0.627						
S35H, G66D, Q99R	M32L, M50D, N52S, M88Q	39.1	2.80E+05	6.57E-04	2.34E-09	1	0.094						
S35H, G66D, Q99H	M32L, M50D, N52S, M88Q	31	7.98E+05	4.41E-03	5.52E-09	5.2	0.207						
S35H, G66D, Q99G	M32L, M50D, N52S, M88Q	790.1	3.00E+06	7.74E-03	2.58E-09	174.8	1.22						
S35H, G66D, Q99I	M32L, M50D, N52S, M88Q	-1.8	5.41E+06	2.06E-04	3.81E-11	0.4	0.361						
S35H, G66D, Q99D	M32L, M50D, N52S, M88Q	399.6	2.57E+10	9.26E+01	3.61E-09	50.8	2.88						
S35H, G66D, Q99A	M32L, M50D, N52S, M88Q	574.6	1.47E+06	4.50E-03	3.07E-09	116.1	0.343						
S35H, G66D, Q99K	M32L, M50D, N52S, M88Q	11.3	3.97E+06	1.46E-03	3.68E-10	0.4	0.111						
S35H, G66D, Q99Y	M32L, M50D, N52S, M88Q	66.3	2.17E+06	5.47E-03	2.51E-09	4.5	0.0663						
S35H, G66D, Q99T	M32L, M50D, N52S, M88Q	854.1	7.52E+06	4.90E-02	6.51E-09	152.2	4.72						
[redacted]													
S35H, G66D, L100P	M32L, M50D, N52S, M88Q	564.1	7.59E+05	3.54E-03	4.66E-09	100.6	14						
S35H, G66D, L100I	M32L, M50D, N52S, M88Q	557.6	1.91E+06	7.12E-04	3.72E-10	108	0.361						
S35H, G66D, L100Y	M32L, M50D, N52S, M88Q	491.9	8.58E+05	8.01E-04	9.34E-10	91.4	0.104						
S35H, G66D, L100H	M32L, M50D, N52S, M88Q	618.7	1.55E+06	1.29E-03	8.30E-10	132.4	0.381						
S35H, G66D, L100W	M32L, M50D, N52S, M88Q	456.4	6.25E+05	1.24E-03	1.98E-09	81.8	0.525						
S35H, G66D, L100R	M32L, M50D, N52S, M88Q	732.6	1.32E+06	1.16E-03	8.78E-10	136.9	0.0885						
S35H, G66D, L100E	M32L, M50D, N52S, M88Q	563.5	2.05E+06	1.45E-03	7.04E-10	112.8	0.197						
S35H, G66D, L100D	M32L, M50D, N52S, M88Q	454.2	5.77E+06	2.02E-03	3.50E-10	97.1	0.22						
S35H, G66D, L100A	M32L, M50D, N52S, M88Q	1033.5	5.82E+07	4.86E-03	8.34E-11	226.9	2.56	60.7	3.72E+06	4.06E-04	1.09E-10	11.3	0.139
S35H, G66D, L100S	M32L, M50D, N52S, M88Q	1088.2	8.03E+06	9.13E-04	1.14E-10	229.1	1.17	86.7	3.61E+06	4.24E-04	1.17E-10	15.2	0.16
S35H, G66D, L100G	M32L, M50D, N52S, M88Q	1193.9	6.87E+06	1.60E-03	2.33E-10	253.1	1.37	63.2	2.76E+06	6.94E-04	2.51E-10	12.6	0.153
S35H, G66D, L100T	M32L, M50D, N52S, M88Q	939.8	1.36E+07	2.78E-03	2.04E-10	202.7	2.13	62.1	1.83E+06	4.81E-04	2.63E-10	11.2	0.14
S35H, G66D, L100V	M32L, M50D, N52S, M88Q	985.4	5.01E+06	9.22E-04	1.84E-10	201	0.741	86.5	1.62E+06	3.85E-04	2.38E-10	14.2	0.16
S35H, G66D, L100Q	M32L, M50D, N52S, M88Q	1087.6	4.97E+06	7.34E-04	1.48E-10	229.9	0.807	66.8	2.26E+06	3.36E-04	1.49E-10	12.9	0.152
S35H, G66D, L100K	M32L, M50D, N52S, M88Q	1132.4	4.29E+06	8.22E-04	1.91E-10	233.6	1.71	88.8	1.49E+06	2.79E-04	1.88E-10	15.1	0.165
S35H, G66D, L100F	M32L, M50D, N52S, M88Q	877.1	1.46E+06	6.14E-04	4.22E-10	168.2	0.231						

[0641] Binding kinetics of these mutant molecules to IL-6 were measured at 37 °C using a Biacore T200. Antibodies were captured on a Protein A chip (GE) by injecting 1:20 cell supernatant dilutions at 10 µL/min for 60 seconds. Five concentrations (0.56, 1.67, 5, 15 and 45 nM) of IL-6 were flowed over captured antibodies for 60 seconds at a flow rate of 30 µL/min and dissociated for 180 seconds in a single cycle kinetics Biacore assay format. The sensor chip was regenerated by a 60 seconds injection of 10 mM Glycine pH 1.7 at the flow rate of 50 µL/min. A similar assay setup was used to monitor interactions between purified antibody samples and IL-6 at 37 °C, when applicable. For this setup, 1 µg/mL of purified antibody was injected over a Protein A chip at 10 µL/min for 25 seconds. Five concentrations (0.56, 1.67, 5, 15 and 45 nM) of IL-6 were flowed over captured antibodies for 60 seconds at the flow rate of 30 µL/min and dissociated for 1800 seconds in a single cycle kinetics Biacore assay format. The sensor chip was regenerated by a 60 seconds injection of 10 mM Glycine pH 1.7 at flow rate of 50 µL/min. Data analysis for supernatant and purified samples were performed using BIAevaluation software (GE). All sensorgrams were double reference subtracted and fit using a 1:1 Langmuir binding model.

[0642] As shown by Tables 18.1 and 18.2, S35H G66D double mutant heavy chain presented a boost on protein expression, as indicated by the approximate two-fold increase in the capture level relative to the wild-type heavy chain. This mutant also presented a small increase in affinity depending on the paired light chain (wild-type: ~2 fold, M32L/M50D/N52S/M88Q: ~1.3 fold). Table 18.1 illustrates G66D optimization of LC wild type Biacore kinetics for Anti-IL-6 heavy chain double mutants. Data were obtained for samples prior (sup) and after purification, both at 37 °C. Table 18.2 illustrates G66D optimization of LC mutant Biacore kinetics for Anti-IL-6 heavy chain double mutants paired with mutagenized light chain. Data were obtained for samples prior (sup) and after purification both at 37 °C.

[0643] Therefore, this double mutant was selected as a starting point for another round where substitution-tolerant positions Q99 and L100 at CDR3 were randomly mutagenized, expressed, and evaluated for IL-6 binding as described for the G66D screening round. As shown in Tables 18.3 and 18.4, L100 substitutions for Ala, Ser, Gly, Thr, Gln, Lys presented superior expression when compared to wild-type as indicated by the capture levels. These mutants were therefore selected for purification and IL-6 binding evaluation. Affinity measurements with the purified material also showed superior IL-6 binding, as illustrated in Tables 18.3 and 18.4. Based

on these results, these additional modified CDRs, as illustrated in FIG 18, were incorporated into the design of the anti-IL-6 and anti-VEGF dual inhibitor molecules.

[0644] Table 18.3 illustrates G66D/S35H heavy chain modification paired with LC wild-type Biacore kinetics for Anti-IL-6 heavy chain triple mutants. Data were obtained for samples prior (sup) and after purification, both at 37 °C. Table 18.4 illustrates G66D/S35H heavy chain modification paired with LC mutant Biacore kinetics for Anti-IL-6 heavy chain triple mutants paired with mutagenized light chain. Data were obtained for samples prior (sup) and after purification, both at 37 °C.

Example 19– Anti-IL-6 Anti-VEGF Dual Inhibitor Variants

[0645] Based on the results with the VEGF trap variants and superior anti-IL-6 paratopes, 216 molecules in two different configurations were designed that were comprised of combinations of sequences as displayed in FIGs. 19, 20, 21, 22 and 23. For the first configuration (VEGFR-Anti-IL-6) the VEGF trap, as shown by sequences 1A-1D of FIG. 19 is positioned at the beginning of the protein, followed by a double repeat of a Gly-Gly-Gly-Gly-Ser linker (GS), as shown by sequence 2A of FIG. 20, which connects the trap to the N-terminus of the anti-IL-6 heavy chain, as shown by sequences 3A-3I of FIG. 21. These constructs can be paired with light chains listed as sequences 4A-4C of FIG. 22. In the second configuration (Anti-IL-6-VEGFR), the variable and constant domains of the heavy chain, illustrated by Heavy chain – Fab, sequences 5A-5I of FIG. 23, are connected to the VEGF trap, illustrated by sequences 1A-1D of FIG. 19, via the GS linker, illustrated by sequence 2A of FIG. 20, and then the Fc domain, illustrated by Heavy chain – Fc, sequences 5A-5I of FIG. 23, is fused to the C-terminal end of the VEGF trap. Thus, the VEGF trap is sandwiched between the antibody Fab and Fc regions.

[0646] FIG. 19 illustrates VEGF trap sequences. The variation in sequence is underlined and highlighted in bold. FIG. 20 illustrates double repeat Gly-Gly-Gly-Gly-Ser linker (GS) sequence. FIG. 21 illustrates heavy chain sequences for Anti-IL-6 molecules. CDRs are underlined. FIG. 22 illustrates light chain sequences for Anti-IL-6 molecules. CDRs are underlined. FIG. 23 illustrates heavy chain (separated into Fab and Fc) sequences for Anti-IL-6 molecules. CDRs are underlined.

[0647] In some embodiments, any of the options of the VEGF trap, wherein it is sandwiched between the antibody Fab and Fc regions, can be paired with light chains listed on

sequences 4A-4C of FIG. 22. Thus, in some embodiments, the constructs outlined in FIG. 19 (the VEGFR sequences) can be used for any of the VEGF trap arrangements provided herein, or on their own.

Example 20– Binding Kinetics between VEGFR-trap Fc fusion and VEGF

[0648] Anti-IL-6Anti-IL-6FIGs. 26A-26C illustrate the results for Biacore assays used to determine the affinity between Anti-VEGF agents and VEGF. Binding kinetics were measured using a Biacore T200 at 37 °C in buffer HBS-EP+ containing 1 mg/mL BSA. VEGFR-Fc (1 µg/mL) and Eylea (1 µg/mL) were captured on a Protein A chip (GE) at 10 µL/min for 25 seconds, five concentrations of VEGF-A165 (0.19, 0.56, 1.67, 5, 15 nM) were flowed over captured anti-VEGF molecules for 120 seconds at 30 µL/min and dissociated for 30 min. The sensor chip surface was regenerated by 60 seconds injection of 10 mM Glycine, pH 1.7 at a flow rate of 50 µl/min. All sensorgrams were double reference subtracted and fit using a 1:1 Langmuir binding model. As shown in FIGs. 26A-26C, VEGFR-Fc and Eylea bind with similar affinity to VEGF. In this example, VEGFR-Fc contains sequence 6A.

Example 21--Removing cleavage product

[0649] As discussed in Example 13, VEGFR-AntiIL6 presents a minor degree of cleavage at the VEGFR1 domain 2 at the VEGF trap region. We developed two orthogonal chromatography methods to separate intact protein from cleaved material after the protein A purification step: i) cation exchange (CEX) and ii) hydrophobic interaction (HIC).

[0650] For the CEX method, VEGFR-AntiIL6 protein A eluate pH solution is adjusted to 6, which is then injected into a Porous XS column pre-equilibrated with 20 mM sodium phosphate pH 6. Intact protein eluted at lower ionic strength than cleaved material and is almost entirely separated within a linear gradient of increasing ionic strength (0-1 M sodium chloride) (**FIG. 32**). Fractions enriched on intact protein were pooled and the overall recovery yield was approximately 57%.

[0651] For the HIC method, a mixture of VEGFR-AntiIL6 intact protein enriched with cleaved material was injected into a Butyl HP column pre-equilibrated with 20 mM sodium phosphate pH 6, 500 mM-1 M ammonium sulfate. Intact and cleaved proteins were separated

within a linear gradient of decreasing ionic strength (1-0 M ammonium sulfate), where intact protein eluted at lower ionic strength than cleaved material and could be partially or completely separated depending on the fractions pooled (**FIG. 33**).

Example 22--VEGFR AntiIL6 affinity to IL6 and VEGF-A measured by KinExA

[0652] The Kinetic Exclusion Assay (KinExA®) 3200 system (Sapidyne Instruments Inc., Boise, ID) was used to measure the equilibrium binding affinity and kinetics between VEGFR-AntiIL6 or VEGFR-AntiIL6-OG1802 (the protein component of which is shown in FIG. 27) and VEGF or IL6 in solution. Azlactone beads coated with VEGF or IL6 were used to capture a fraction of free VEGFR-AntiIL6 or VEGFR-AntiIL6-OG1802 from an equilibrated sample of VEGFR-AntiIL6 or VEGFR-AntiIL6-OG1802 and VEGF or IL6. Captured dual inhibitor molecules were detected with a fluorescent labeled polyclonal anti-human antibody (Alexa 647 Goat, anti-human IgG).

[0653] Affinity between dual inhibitor molecules and VEGF were determined from 2-3 titration curves at increasing dual inhibitor concentrations and their corresponding VEGF titrating serial dilutions (indicated in parenthesis) listed as follow: I) VEGFR-AntiIL6: 1 nM (20 nM, 2-fold dilution, 12 dilutions) 3.5 hours incubation; 50 pM (500 pM, 2-fold dilution, 14 dilutions) 2.5 hours incubation; 10 pM (1 nM, 2-fold dilution, 13 dilutions) 23 hours incubation; II) VEGFR-AntiIL6-OG1802: 2 nM (30 nM, 2-fold dilution, 12 dilutions) 45 min incubation; 100 pM (15 nM, 2-fold dilution, 14 dilutions) 6 hours incubation; 7.5 pM (1 nM, 2-fold dilution, 14 dilutions) 48 hours incubation. Similar measurements were performed with IL6 under the following conditions: III) VEGFR-AntiIL6: 1 nM (20 nM, 2-fold dilution, 13 dilutions) 7 hours incubation; 10 pM (1 nM, 2-fold dilution, 13 dilutions) 42 hours incubation; 100 pM (1 nM, 2-fold dilution, 13 dilutions) 2.5 hours incubation; IV) VEGFR-AntiIL6-OG1802: 1 nM (22.5 nM, 2-fold dilution, 13 dilutions) 5.5 hours incubation; 100 pM (22.5 nM, 2-fold dilution, 14 dilutions) 12 hours incubation.

[0654] On rates (k_{on}, $M^{-1}s^{-1}$) between dual inhibitor molecules and VEGF or IL6 were directly determined from kinetic measurement experiments (1-2 curves for each analyzed pair), while off rates (k_{off}, s^{-1}) were calculated based on the following equation: $k_{off} = K_d \times k_{on}$. Measurements were conducted on four preparations containing i) 100 pM VEGFR-AntiIL6 and

86.4 pM VEGF, ii) 217 pM VEGFR-AntiIL6-OG1802 and 200 pM VEGF, iii) 100 pM VEGFR-AntiIL6 and 84.3 pM IL6, iv) 1000 pM VEGFR-AntiIL6 and 900 pM IL6.

[0655] All measurements were done at 37 °C using 1x PBS running buffer (pH 7.4) and samples prepared in 1xPBS (pH 7.4) with 1 mg/mL BSA. Data analyses were done using KinExA Pro software 4.3.11.

[0656] Under the conditions tested, VEGFR-AntiIL6-OG1802 binds to VEGF and IL6 in a comparable manner to VEGFR-AntiIL6 with Kd values of 2.10 pM to VEGFR-AntiIL6 and VEGF, 1.02 pM to VEGFR-AntiIL6-OG1802 and VEGF, 21.10 pM to VEGFR-AntiIL6 and IL6, 12.10 pM to VEGFR-AntiIL6-OG1802 and IL6 (see table 22.1 and 22.2).

Table 22.1 VEGF

	VEGFR-AntiIL6	VEGFR-AntiIL6-OG1802
Kd (pM)	2.10	1.02
95% confidence interval (pM)	1.34 - 2.91	0.46 - 1.80
On Rate ($M^{-1}s^{-1}$)	4.04×10^{7}	3.75×10^{6}
95% confidence interval	3.31×10^{7} - 4.92×10^{7}	2.90×10^{6} - 4.90×10^{6}
Off Rate ($s-^{1}$)	8.49×10^{-5}	3.83×10^{-6}

Table 22.2 –IL-6

	VEGFR-AntiIL6	VEGFR-AntiIL6-OG1802
Kd (pM)	21.10	12.10
95% confidence interval (pM)	14.30 - 30.80	8.47 - 16.30
On Rate ($M^{-1}s^{-1}$)	1.15×10^{7}	8.00×10^{6}
95% confidence interval	9.57×10^{6} - 1.37×10^{7}	6.44×10^{6} - 9.93×10^{6}
Off Rate ($s-^{1}$)	2.42×10^{-4}	9.66×10^{-5}

[0657] The measured affinities of VEGFR-AntiIL6 and VEGFR-AntiIL6-OG1802 were within error for both IL6 and VEGF, thus these results demonstrate that the biopolymer does not affect binding of dual inhibitors to its targets.

Example 23--VEGF/VEGFR Competitive ELISA

[0658] This assay was performed as described in example 3. In this assay, VEGFR-AntiIL6, VEGFR-AntiIL6-OG1802 (as shown in FIG. 27), and Eylea all inhibited VEGF binding

to VEGFR in varying degrees (**FIG. 34**). VEGFR-AntiIL6-OG1802 showed superior inhibition (IC_{50} = 0.99 nM) when compared to VEGFR-Anti-IL6 (IC_{50} = 1.98 nM) and Eylea (IC_{50} = 1.38 nM). Additionally, VEGFR-AntiIL6 showed superior maximal inhibition over Eylea, while VEGFR-AntiIL6-OG1802 had an even more pronounced maximal inhibition (VEGFR-AntiIL6-OG1802 = 79.5%, VEGFR-Anti-IL6 = 67.0%, vs. Eylea = 49.6%). These data indicate that the addition of the biopolymer OG1802 to VEGFR-AntiIL6 creates additional inhibitory abilities for VEGF binding to VEGFR. The biopolymer therefore adds to the function of the molecule.

Example 24--IL-6/IL-6R Competitive ELISA

[0659] This assay was performed as described in example 3.

[0660] Under these conditions, the VEGFR-AntiIL6-OG1802 effectively blocked the IL-6/IL-6R interaction in a manner comparable to VEGFR-AntiIL6 with IC_{50} values of 0.26nM for VEGFR-AntiIL6 and 0.23nM for VEGFR-AntiIL6-OG1802 (**FIG. 35**). These results indicate that the biopolymer does not alter the inhibitory effects of the dual inhibitor on IL6 binding to its receptor. Eylea served as a negative control.

Example 25--Cell based VEGF stimulated VEGFR reporter assay:

[0661] This assay was performed as described in example 15.

[0662] Under the conditions tested, VEGFR-AntiIL6-OG1802 inhibited VEGF stimulated VEGFR reporter cells in a comparable manner to VEGFR-AntiIL6 and Eylea with average IC_{50} values of 0.35±0.10nM, 0.51±0.07nM, and 0.35±0.03nM, respectively (**FIG. 36**). There may be a trend that VEGR-AntiIL6-OG1802 (the protein component as shown in **FIG. 27**) and Eylea are moderately more efficacious than VEGFR-AntiIL6, but this is not a significant finding and falls within the error of the assay. We can conclude form these data that the bioconjugate inhibits VEGFR2 signaling similarly to its protein precursor.

Example 26--Lipopolysaccharide stimulated tubule formation in HUVECs:

[0663] Passage 2 HUVEC cells were cultured in Vascular Basal Media supplemented with 1x Bovine Brain Extract endothelial cell growth kit (ATCC; growth media) in 5% CO_2 at 37

degrees. Once cells reached 70% confluency, cells were lifted from the plate using trypsin (0.05%; ATCC) and 0.75x10^6 cells plated in a T25 flask in growth media.

[0664] The next day, media was swapped for Assay Media (2% FBS in Vascular Basal Medium). After 6 hours, 5µg/ml of Calcein AM was added directly to the media and incubated for 30 minutes at 37 degrees.

[0665] At the same time, 200µls of prethawed Matrigel (growth factor reduced; Corning) was added to each well of a 8-well glass chamber slide and incubated at 37 degrees for 30 minutes.

[0666] During incubations, inhibitors (37.5nM final concentration) were mixed with lipopolysaccharide (LPS; Sigma; 1ug/ml final concentration).

[0667] Following the 30 minute Calcein AM incubation, the cells were washed 3x with PBS and then trypsinized. Harvested cells were centrifuged for 3 minutes at 1,500xg and resuspended in assay medium at a density of 500,000 cells/ml. 150µls of cells were then mixed with 100µls of prepared inhibitor/complex solutions, and then transferred to the Matrigel coated chamber slides and incubated overnight.

[0668] The next day, tubules were imaged using a 10x objective lens and the GFP filter on a EVOS fluorescence microscope (Life Technologies). At least 2 representative images were taken for each condition and then analyzed using the Angiogenesis Analyzer Plugin for ImageJ (NIH), which evaluates and quantifies 20 tubule network formation parameters.

[0669] Statistical significance was determined from all replicates from independent experiments using a unpaired, two-tailed Students t-test.

[0670] Under these conditions, VEGFR-AntiIL6 showed statistically significant inhibition of 15/20 measured angiogenic parameters over control, while Eylea significantly inhibited 5/20 and anti-IL6 inhibited 3/20 relative to control treated cells. Altogether, all but one of the parameters that trended or were significantly affected by Eylea or anti-IL6 were significantly inhibited by VEGFR-AntiIL6. Additionally, there were several parameters where VEGFR-AntiIL6 displayed significantly better inhibition over Eylea (7/20) and anti-IL6 (10/20), 3 of which VEGFR-AntiIL6 was significantly better than both Eylea and anti-IL6. (FIG. 37 and FIG. 38 and 11)

[0671] VEGFR-AntiIL6-OG1802 effectively inhibited HUVEC tubule formation, and interestingly, VEGFR-AntiIL6-OG1802 showed a trend of increased inhibition in 9/20 parameters

over VEGFR-AntiIL6. (FIG. 39). This implies that VEGFR-AntiIL6-OG1802 has added benefit over its unconjugated counterpart.

Example 27--HUVEC proliferation assay:

[0672] This assay was performed as described in example 17.

[0673] Under the conditions tested, VEGFR-AntiIL6-OG1802 (the protein component as shown in **FIG. 27**) inhibited VEGF/IL6 stimulated HUVEC proliferation in a comparable manner to VEGFR-AntiIL6, while Eylea had no effect (**FIG. 40A**).

[0674] Interestingly, VEGFR-AntiIL6-OG1802 showed increased efficacy relative to VEGFR-AntiIL6 when different densities of cells were seeded per well at the beginning of the experiment. As shown in **FIG. 40B**, at 2,000 cells per well, VEGFR-AntiIL6 and VEGFR-AntiIL6-OG1802 showed nearly identical inhibition of HUVEC proliferation. At 4,000 cells per well, VEGFR-AntiIL6-OG1802 showed slightly better maximal inhibition than VEGFR-AntiIL6 (40% vs. 30%, respectively), and at 6,000 cells per well, VEGFR-AntiIL6-OG1802 inhibited proliferation by 25% relative to control, while VEGFR-AntiIL6 had no effect.

[0675] As shown in the examples above, without intending to be limited by theory, it appears that the biopolymer helps deliver its protein counterpart to the surface of the plate and cells. This allows the drug to primarily inhibit the pools of VEGF and IL6 that are most likely to activate cell signaling and avoid being saturated by VEGF and IL6 suspended higher in the fluid phase and less likely to affect cell signaling. As cell numbers increase, this effect would be more pronounced as more drug is needed to act on more cells. Thus, in some embodiments, the conjugate provides a superior product in that it can assist in distribution of the molecule.

[0676] All patent filings, websites, other publications, accession numbers and the like cited above or below are incorporated by reference in their entirety for all purposes to the same extent as if each individual item were specifically and individually indicated to be so incorporated by reference. If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing

date of a priority application referring to the accession number if applicable. Likewise if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect of the invention can be used in combination with any other unless specifically indicated otherwise. Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

What is claimed is:

~~1. An isolated antagonist antibody that specifically binds to IL-6 that is conjugated to a polymer.~~

~~2. An isolated antagonistic IL-6 antibody comprising:~~

~~a heavy chain amino acid variable region that comprises a heavy chain that has a sequence of at least one of SEQ ID NOs: 7-13,19-27, 89, 90, 256-262; and~~

~~a light chain amino acid variable region that comprises the light chain that has a sequences of at least one of SEQ ID NOs: 91-93, 28-30.~~

~~3. An isolated antagonist IL-6 antibody comprising: a heavy chain variable region (VH) comprising 3 complementarity determining regions: VH (CDR1), VH CDR2, and VH CDR3 having an amino acid sequence from the CDRs listed in SEQ ID NO: 256; and a light chain variable region (VL) comprising a VL CDR1, VL CDR2, and VL CDR3 having an amino acid sequence selected from the group of CDRs listed in SEQ ID NO: 91-93.~~

~~4. An isolated antagonist antibody that binds to IL-6, wherein the antibody comprises at least one of the following mutations based on EU numbering: L234A, L235A, and G237A.~~

~~5. An isolated antagonistic antibody that binds to IL-6, the antibody comprising:~~

~~a CDR_H1 that is a CDR_H1 in SEQ ID NO: 172;~~

~~a CDR_H2 that is a CDR_H2 in SEQ ID NO: 173;~~

~~a CDR_H3 that is a CDR_H3 in SEQ ID NO: 174:~~

~~a CDR_L1 that is a CDR_L1 in SEQ ID NO: 199;~~

~~a CDR_L2 that is a CDR_L2 in SEQ ID NO: 200;~~

~~a CDR_L3 that is a CDR_L3 in SEQ ID NO: 201;~~

~~at least one of the following mutations (EU numbering): L234A, L235A, and G237A; and~~

~~at least one of the following mutations (EU numbering): Q347C or L443C.~~

~~6. The isolated antagonist antibody of any one of claims 1 to 6, wherein the antibody binds human IL-6 with an affinity of between about 10 pM to about 1 nM.~~

~~7. The isolated antagonist antibody of any one of claims 1-6, wherein the heavy chain constant domain further comprises a cysteine residue introduced by recombinant DNA technology.~~

~~8. The isolated antagonist antibody of any one of claims 1-7, wherein the cysteine residue is selected from the group consisting of Q347C and L443C (EU numbering).~~

~~9. The isolated antagonist antibody of any one of claims 1-8, wherein the cysteine residue is L443C (EU numbering).~~

~~10.~~1. A fusion protein comprising:

~~the~~ an isolated antagonist antibody that specifically binds to IL-6~~of any one of claims 1-9~~; and

a VEGF Trap~~.~~,

~~11. The fusion protein of claim 10,~~ wherein the VEGF Trap is positioned either

~~to~~ at an N-terminal end of a heavy chain comprising IL-6 VH; or

between a hinge region and ~~after~~ a CH1 domain of ~~a~~ the heavy chain comprising IL-6 VH.

~~12.~~ 2. The fusion protein of claim ~~11~~1, wherein the VEGF Trap is at an N-terminal end of the heavy chain comprising IL-6 VH.

~~13.~~ 3. The fusion protein of claim ~~11~~1, wherein the VEGF Trap is between the hinge region and after a CH1 domain of the heavy chain comprising IL-6 VH.

~~14.~~ 4. The fusion protein of any one of claims ~~10~~1-3, wherein the VEGF Trap ~~binding domain~~ comprises at least one extracellular VEGF binding domain from at least two of VEGFR-1, -2, or -3.

5. The fusion protein of any one of claims 1-4, wherein the VEGF Trap comprises VEGFR1 domain 2 and VEGFR2 domain 3.

~~15.~~ 6. The fusion protein of claim ~~14~~5, wherein the VEGF Trap ~~binding domain~~ consists of the sequence of SEQ ID NO: 114.

7. The fusion protein of any one of claims 1-6, wherein the antibody binds human IL-6 with an affinity of between about 10 pM to about 1 nM.

8. The fusion protein of any one of claims 1-7, comprising a heavy chain constant domain comprising a non-native cysteine residue.

9. The fusion protein of claim 8, wherein the non-native cysteine residue is at a position selected from the group consisting of 347 and 443, EU numbering.

10. The fusion protein of claim 9, wherein the non-native cysteine residue is at position 443, EU numbering.

~~16. The fusion protein of claim 14, wherein the VEGF binding domain works as a VEGF trap, preventing VEGF from binding to cellularly expressed VEGF receptors.~~

~~17. A conjugate comprising:~~

~~(a) any of the isolated antagonistic antibodies from claims 1 to 9; and~~

~~(a) a polymer, wherein the polymer is covalently attached to the antibody.~~

~~18. The conjugate of claim 17, wherein the polymer comprises a phosphorylcholine containing polymer.~~

~~19. The conjugate of claim 17, wherein the polymer comprises a zwitterionic monomer, wherein the zwitterionic monomer is selected from the group consisting of HEMA-phosphorylcholine, PEG, biocompatible fatty acids and derivatives thereof, Hydroxy Alkyl Starch (HAS), Hydroxy Ethyl Starch (HES), Poly Ethylene Glycol (PEG), Poly (Gly_x-Ser_y) (HAP), Hyaluronic acid (HA), Heparosan polymers (HEP), Fleximers, Dextran, Poly-sialic acids (PSA), Fc domains, Transferrin, 25 Albumin, Elastin like (ELP) peptides, XTEN polymers, PAS polymers, PA polymers, Albumin binding peptides, CTP peptides, and FcRn binding peptides.~~

~~20.~~ 11. A conjugate comprising:

the fusion protein of any one of claims ~~10-16~~1-10; and

a polymer, wherein the polymer is covalently attached to a heavy chain of the isolated antagonist antibody~~fusion protein~~.

~~21.~~12. The conjugate of claim ~~20,~~11 wherein the polymer comprises a phosphorylcholine containing polymer.

~~22.~~13. The conjugate of claim ~~20~~11, wherein the polymer comprises a zwitterionic monomer, wherein the zwitterionic monomer is selected from the group consisting of HEMA-phosphorylcholine, PEG, biocompatible fatty acids and derivatives thereof, Hydroxy Alkyl Starch (HAS), Hydroxy Ethyl Starch (HES), Poly Ethylene Glycol (PEG), Poly (Gly_x-Ser_y) (HAP), Hyaluronic acid (HA), Heparosan polymers (HEP), Fleximers, Dextran, Poly-sialic acids (PSA), Fc domains, Transferrin, 25 Albumin, Elastin like (ELP) peptides, XTEN polymers, PAS polymers, PA polymers, Albumin binding peptides, CTP peptides, and FcRn binding peptides.

~~23.~~14. The conjugate of any one of claims ~~17-22~~11-13, wherein the polymer comprises 2(methacryloyloxy)ethyl (2-(trimethylammonio)ethyl) phosphate (MPC) monomers.

~~24.~~15. The conjugate of any one of claims ~~17-22~~11-14, wherein the polymer has a peak molecular weight between 300,000 and 1,750,000 Daltons as measured by size exclusion chromatography – multi angle light scattering (hereinafter “SEC-MALS”).

~~25.~~16. The conjugate of any one of claims ~~17~~11-~~24~~15, wherein the polymer has a peak molecular weight between 500,000 and 1,000,000 Daltons as measured by SEC-MALS.

~~26.~~17. The conjugate of claim ~~22~~16, wherein the polymer has a peak molecular weight between 600,000 to 800,000 Daltons as measured by SEC-MALS.

~~27.~~18. The conjugate of any one of claims ~~17~~11-~~26~~17, wherein the polymer has 2 or more arms.

~~28.~~19. The conjugate of any one of claims ~~17-27~~11-18, wherein the polymer has 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms.

~~29.~~20. The conjugate of claim ~~28~~19, wherein the polymer has 9 arms.

~~30.~~21. The conjugate of any one of claims ~~17-29~~11-20, wherein the antibody is an IgG.

~~31.~~22. The conjugate of claim ~~30~~21, wherein the polymer is covalently bonded to a sulfhydryl group from a cysteine residue on the IgG heavy chain.

~~32. The conjugate of any one of claims 17-31, wherein the antibody comprises a cysteine residue at position 347 or 443 (EU numbering).~~

~~33.~~23. The conjugate of any one of claims ~~32~~11-22, wherein the polymer is covalently bonded to a sulfhydryl group from a cysteine residue at position 347 or 443, ~~(~~EU numbering~~)~~.

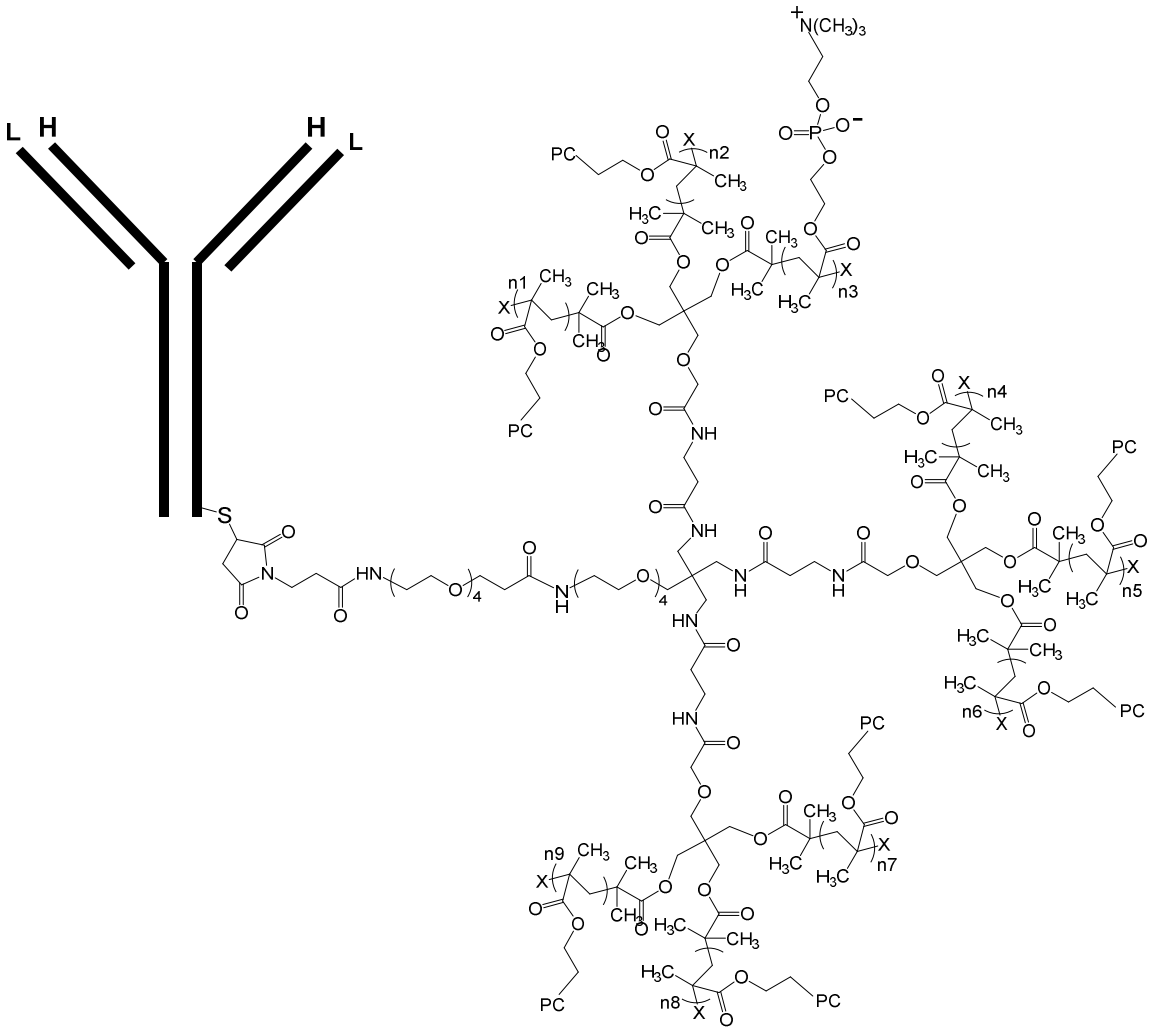
~~34.~~24. The conjugate of any one of claims ~~17-33~~11-23, wherein the antibody is further conjugated to a polymer to form a bioconjugate, and wherein the bioconjugate has a molecular weight between about 350,000 and 1,900,000 Daltons.

~~35.~~25. The conjugate any one of claims ~~17-34~~11-24, wherein the PolyDispersity Index (PDI) is equal to or less than 1.5.

~~36.~~26. The conjugate of any one of claims ~~17-35~~11-25, wherein the polymer has 9 arms; and

the polymer has a molecular weight of between about 600,000 to about 900,000 Da.

~~37.~~27. The conjugate of any one of claims ~~17-36~~11-26, which comprises the following structure:

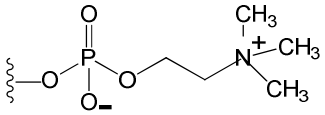


Formula (17)

wherein:

each heavy chain of the anti-IL-6 antibody is denoted by the letter H, and each light chain of the anti-IL-6 antibody is denoted by the letter L;

the polymer is bonded to the antibody through the sulfhydryl of C443, ~~(~~EU numbering~~)~~, which bond is depicted on one of the heavy chains;

PC is  , where the curvy line indicates the point of attachment to the rest of the polymer, where X=a) OR where R=H, methyl, ethyl, propyl, isopropyl, b) H, or c) any halide, including Br; and

n1, n2, n3, n4, n5, n6, n7, n8 and n9 are the same or different such that the sum of n1, n2, n3, n4, n5, n6, ~~n6,~~ n7, n8 and n9 is 2500 plus or minus 15%,

wherein ~~if the conjugate comprises a VEGF Trap,~~ the VEGF Trap is fused:

to the N-terminal end of the heavy chain; or

between a hinge region and a Fab region (after the CH1 domain) of the heavy chain.

~~38.~~ 28. The conjugate of claim ~~37~~27, wherein ~~if~~ the ~~conjugate comprises a~~ VEGF Trap_~~, it~~ comprises ~~a~~from N-to-C terminus the VEGFR1 domain 2 and then ~~a~~ the VEGFR2-Domain 3~~, in that order~~.

~~39.~~ 29. An isolated cell line that produces ~~an isolated antagonistic antibody of any one of claims 1-9 or~~ the fusion protein of any one of claims ~~10-16~~1-10.

~~40.~~ 30. The isolated cell line of claim ~~39~~29, wherein the cell line is selected from the group consisting of CHO, k1SV, XCeed, CHOK1SV, and GS-KO.

~~41.~~ 31. An isolated nucleic acid encoding ~~an isolated antagonistic antibody of any one of claims 1-9 or~~ the fusion protein of any one of claims ~~10-16~~1-10.

~~42.~~ 32. A recombinant expression vector comprising the nucleic acid of claim ~~41~~31.

~~43.~~ 33. A host cell comprising the expression vector of claim ~~42~~32.

~~44.~~ 34. A method of producing ~~an IL-6 antagonist antibody or~~a fusion protein~~ thereof~~, the method comprising: culturing a cell line that recombinantly produces ~~an isolated antagonistic antibody of any one of 1-9 or~~ the fusion protein of any one of claims ~~10-16~~1-10 under conditions wherein the antibody is produced; and recovering the antibody.

~~45.~~ 35. A pharmaceutical composition comprising ~~an isolated antagonistic antibody of any one of claims 1-9 or~~ the fusion protein of any one of claims ~~10-16~~1-10 and/or ~~a~~ the conjugate of any one of claims ~~17~~11-~~38~~28, and a pharmaceutically acceptable carrier.

~~46.~~ 36. The pharmaceutical composition of claim ~~45~~35, wherein the composition is a liquid and has an endotoxin level less than about 0.2 EU/ml.

~~47.~~ 37. The pharmaceutical composition of claim ~~46~~36, wherein the composition is a liquid and has an endotoxin level less than about 2.0, 1, 0.5, or 0.2 EU/ml.

~~48.~~ 38. Use of ~~A method for the treatment or prophylaxis of a disease in a patient in need thereof, said method comprising administering to the patient an isolated antagonist antibody of any claims 1-9 or~~ the fusion protein of any one of claims ~~10-16~~1-10 and/or ~~a~~ the conjugate of any one of claims ~~17-38~~11-28 in the manufacture of a medicament for the treatment or prophylaxis of a neovascular retinal disease in a patient in need thereof.

~~49.~~ 39. ~~A method for the treatment or prophylaxis of a disease in a patient in need thereof, said method comprising:~~

~~identifying a patient having hyperactive IL-6 and/or VEGF activity; and~~

~~administering to the patient an isolated antagonist antibody of any one of claims 1-9 or~~Use of the fusion protein of any one of claims ~~10-16~~1-10 and/or ~~a~~ the conjugate of any one of claims ~~17-38~~11-28 in

the manufacture of a medicament for the treatment or prophylaxis of an ocular disorder in a patient in need thereof.

~~50. The method of claim 49, wherein the disease is an ocular disorder.~~

~~51.~~ 40. The use~~method~~ of claim ~~50~~39, wherein the ocular disorder is at least one of wet or neovascular macular degeneration, wet or neovascular age-related macular degeneration, dry age-related macular degeneration, venous, arterial, or other blockage of the ocular and or retinal blood vessels with or without retinal edema, anterior and posterior uveitis, uveitic macular edema, diabetic retinopathy, non-proliferative diabetic retinopathy, diabetic macular edema, non-proliferative diabetic macular edema vention, ophthalmic inflammatory disease scleritis and intraocular tumors.

~~52.~~ 41. The use ~~method~~ of any one of claims ~~49-51~~38-40, wherein the ~~isolated antibody, fusion protein, or the conjugate~~medicament is to be administered no more frequently than once a month.

~~53.~~ 42. The use~~method~~ of any one of claims 38-40~~49-51~~, wherein the medicament~~isolated antibody, fusion protein, or the conjugate~~ is to be administered no more frequently than once every two months.

~~54.~~ 43. The use~~method~~ of any one of claims 38-40~~49-51~~, wherein the ~~isolated antibody, fusion protein, or the conjugate~~medicament is to be administered no more frequently than once every three months.

~~55.~~ 44. The use~~method~~ of any one of claims 38-40~~49-54~~, wherein the ~~isolated antibody, fusion protein, or the conjugate~~medicament is to be administered with a frequency between once a year and once every two months.

~~56.A fusion protein comprising:~~

~~an IL-6 VH~~

~~an IL-6 VL~~

~~an IL-6 Fc;~~

~~a VEGF Trap,~~

~~wherein the VEGF Trap is fused to IL-6 in one of the following manners:~~

~~to an N-terminal end of a heavy chain comprising IL-6 VH; between a hinge region and after a CH1 domain of a heavy chain comprising IL-6 VH.~~

~~57. The fusion protein of claim 56, wherein if the conjugate comprises a VEGF Trap, it comprises a VEGFR1 domain 2 and then a VEGFR2-Domain 3, in that order.~~

~~58. The fusion protein of any one of the preceding claims, comprising a mutation at position 94 in the VEGF Trap sequence.~~

~~59. The fusion protein of any one of the preceding claims, comprising a mutation at position 95 in the VEGF Trap sequence.~~

~~60. The fusion protein of any one of the preceding claims, comprising mutations of T94I and/or H95I in the VEGF Trap sequence.~~

~~61. A VEGFR-Anti-IL-6 dual inhibitor, wherein the VEGFR-Anti-IL-6 dual inhibitor comprises a trap antibody fusion of an anti-IL 6 antibody and an anti-VEGF trap (VEGFR1/2), wherein the dual inhibitor includes at least one point mutation within a VEGFR sequence to reduce cleavage of the VEGFR protein.~~

~~62. The VEGFR-Anti-IL-6 dual inhibitor of Claim 61, wherein a molecular weight of the VEGFR-anti-IL-6 dual inhibitor is 1.0 MDa.~~

~~63. The VEGR-Anti-IL-6 dual inhibitor of Claim 61, wherein the VEGFR-Anti-IL-6 dual inhibitor provides therapy for inflammatory retinal diseases.~~

~~64. The VEGFR-Anti-IL-6 dual inhibitor of Claim 61, comprising a constant heavy, a constant light, a fragment antigen binding, a fragment crystallizable (Fc), a vascular endothelial growth factor receptor (VEGFR), a variable heavy, and a variable light regions.~~

~~65. The VEGFR-Anti-IL-6 dual inhibitor of Claim 64, wherein the Anti-IL-6 heavy chain variable region sequences is selected from options SEQ ID NO: 7-13, 89, 90, and/or 256-262 .~~

~~66. The VEGFR-Anti-IL-6 dual inhibitor of any one of claims 64-65, wherein the VEGF trap sequences is selected from at least one of SEQ ID Nos: 145, 15, 16, or 17.~~

~~67. The VEGFR-Anti-IL-6 dual inhibitor of any one of claims 64-66, wherein the linker sequence is SEQ ID NO: 18.~~

~~68. The VEGR-Anti-IL-6 dual inhibitor of any one of claims 64-67, wherein the heavy chain sequence for Anti-IL-6 molecules is selected from at least one of SEQ ID NOs 19-27 or includes at least the sequence in one of SEQ ID NOs: 89, 90, 256-262.~~

~~69. The VEGFR-Anti-IL-6 dual inhibitor of any one of claims 64-68, wherein the light chain sequence for Anti-IL-6 molecules comprises at least 1, 2, or 3 light chain CDRs from at least one of SEQ ID NOs 76-84.~~

~~70. The VEGFR-Anti-IL-6 dual inhibitor of any one of claims 64-69, wherein the heavy chain sequence for the Anti-IL-6 molecule comprises at least 1, 2, or 3 heavy chain CDRs from at least one of SEQ ID NOs 49-75.~~

~~71. The VEGFR-Anti-IL-6 dual inhibitor of any one of claims 64-70, comprising a VEGFR-Fc sequence from at least one of SEQ ID NOs 85-88.~~

~~72.The VEGFR-Anti-IL-6 dual inhibitor of any one of claims 64-71, comprising one or more of the sequences in any one or more of SEQ ID Nos 7-13, 145, 15-17, 18-84.~~

~~73.The VEGFR-Anti-IL-6 dual inhibitor of claim 72, comprising:~~

~~an IL-6 VH;~~

~~an IL-6 VL;~~

~~an IL-6 Fc;~~

~~a VEGF Trap; and~~

~~a linker.~~

~~74. The VEGFR-Anti-IL-6 dual inhibitor of claim 73, wherein the IL-6 VH comprises a sequence from an IL6 VH sequence in any one of SEQ ID NOs 19-27, 31-39, 89, 90, or 256-262.~~

~~75. The VEGFR-Anti-IL-6 dual inhibitor of claim 73 or 74, wherein the IL-6 VL comprises a sequence from an IL6 VL sequence in any one of SEQ ID Nos 28-30 or 91-93.~~

~~76. The VEGFR-Anti-IL-6 dual inhibitor of any one of claims 73-75, wherein the Fc comprises a sequence from a Fc sequence in any one of SEQ ID NOs 40-48.~~

~~77. The VEGFR-Anti-IL-6 dual inhibitor of any one of claims 73-76, wherein the VEGF Trap comprises a sequence from a VEGF trap sequence in any one of SEQ ID NOs: 145, 15, 16, or 17.~~

~~78. A protein construct comprising:~~

~~at least 3 heavy chain CDRs;~~

~~at least 3 light chain CDRs;~~

~~a VEGF trap sequence; and~~

~~a linker sequence, wherein each of the sequences is selected from a corresponding sequence within FIGs. 18-25.~~

~~79. A fusion protein comprising:~~

~~an IL-6 VH;~~

~~an IL-6 VL;~~

~~an IL-6 Fc; and~~

~~a VEGF Trap,~~

~~wherein the fusion protein alters HUVEC proliferation.~~

~~80. The fusion protein of claim 79, wherein altering HUVEC proliferation is inhibiting VEGF/IL6 mediated proliferation.~~

~~80. A fusion protein comprising a sequence that is at least 80% identical to SEQ ID NO: 263 and at least 80% identical to SEQ ID NO: 117, wherein the fusion protein is further conjugated to a polymer.~~

~~81. The fusion protein of claim 80, wherein the fusion protein is at least 95% identical to SEQ ID NO: 263 and at least 95% identical to SEQ ID NO: 117.~~

~~82. The fusion protein of claim 80 or 81, wherein the protein comprises at least a) SEQ ID NO: 172, 173, 174, 199, 200, and 201, or b) a substitutions of 1, 2, or 3 amino acids within SEQ ID NO: 172, 173, 174, 199, 200, and/or 201, wherein the substitution is a conservative substitution.~~

~~83. An antibody of any one of claims 1-9, wherein the antibody comprises a) an amino acid sequence of SEQ ID NO: 169 and 170 or b) an amino acid sequence that is at least 80% identical to SEQ IDNO: 169 and at least 80% identical to SEQ ID NO: 170.~~

~~84.~~45. A fusion protein of any one of claims ~~10-16~~1-10~~, 56-60, or 79-81~~, wherein the fusion protein comprises a) an amino acid sequence of SEQ ID NO: 169 and 170 or b) an amino acid sequence that is at least 80% identical to SEQ ID NO: 169 and at least 80% identical to SEQ ID NO: 170.

~~85.~~46. A conjugate of any one of claims ~~17-38~~11-28, wherein the ~~conjguate~~conjugate comprises a) an amino acid sequence of SEQ ID NO: 169 and 170 or b) an amino acid sequence that is at least 80% identical to SEQ ID NO: 169 and at least 80% identical to SEQ ID NO: 170.

~~86. A VEGFR-Anti-IL-6 dual inhibitor of any one of claims 61-77, wherein the VEGFR-Anti-IL-6 dual inhibitor comprises a) an amino acid sequence of SEQ ID NO: 169 and 170 or b) an amino acid sequence that is at least 80% identical to SEQ ID NO: 169 and at least 80% identical to SEQ ID NO: 170.~~

~~87. A protein of claim 78, wherein the protein comprises a) an amino acid sequence of SEQ ID NO: 169 and 170 or b) an amino acid sequence that is at least 80% identical to SEQ ID NO: 169 and at least 80% identical to SEQ ID NO: 170.~~