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(54) **Title:** THERAPEUTIC AGENTS WITH INCREASED OCULAR RETENTION

(57) **Abstract:** Modified agents (e.g., modified therapeutic agents) with improved ocular retention are provided. Also provided are methods of making and using the modified agents, e.g., in the treatment of ocular diseases such as macular edema, macular degeneration, and uveitis.

THERAPEUTIC AGENTS WITH INCREASED OCULAR RETENTION

RELATED APPLICATIONS

This application claims priority to **U.S.** Provisional Application No. 62/077,072, filed November 7, 2014, and **U.S.** Provisional Application No. 62/087,942, filed December 5, 2014. The entire contents of each of these applications are incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates to agents that have improved ocular retention.

BACKGROUND

Ocular diseases can be treated by intraocular administration of therapeutic agents. To achieve greater therapeutic efficacy and/or to improve convenience, patient comfort and compliance, it is desirable to provide engineered therapeutic agents for ocular (e.g., intravitreal) administration that have improved retention in the eye. The present invention relates to engineered agents with improved retention in the eye and compositions comprising the engineered agents, as well as methods for producing and using the engineered agents. For example, the engineered agents can be used in the treatment of eye diseases.

SUMMARY

The present invention relates to Applicants' discovery that certain antibodies described herein have surprisingly good retention in the eye. The invention relates to compositions comprising engineered agents that have improved ocular retention, methods of producing compositions comprising engineered agents with improved ocular retention, and uses of such compositions for the treatment of ocular disease.

In one aspect, the invention provides, an ocular half life extending (OHLE) polypeptide. In an embodiment, the OHLE polypeptide is coupled to, e.g., covalently coupled to, an agent, e.g., a therapeutic or diagnostic agent.

In an embodiment, the OHLE polypeptide comprises:

a) a heavy chain module comprising, e.g., in the N terminal to C terminal direction, a heavy chain variable region module and an IgG2 module; and

b) a light chain module comprising a light chain variable region module.

In one embodiment, the heavy chain variable region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with a heavy chain variable region of an antibody in Table 4. In one embodiment, the heavy chain variable region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with SEQ ID NO: 37 or 17.

In one embodiment, the IgG2 constant region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with an IgG2 constant region of an antibody in Table 4. In one embodiment, the IgG2 constant region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with SEQ ID NO: 73 or 74. In other embodiments, the IgG2 constant region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with one or more of SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 51, SEQ ID NO: 66 or SEQ ID NO: 67.

In one embodiment, the light chain variable region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with a light chain variable region of an antibody in Table 4. In one embodiment, the light chain variable region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with SEQ ID NO: 38 or 18.

In one embodiment, the heavy chain module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity to the heavy chain framework regions of SEQ ID NOS: 41 or 47. In one embodiment, the heavy chain module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity to one or more of, e.g., one, two, three, or all of, the heavy chain framework region 1, e.g., HC FR1, heavy chain framework region 2, e.g., HC FR2, heavy chain framework region 3, e.g., HC FR3, or heavy chain framework region 4, e.g., HC FR4. The heavy chain framework regions are depicted in Figures 15A.

In one embodiment, the light chain module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity to the light chain framework regions of SEQ ID NOS: 41 or 47. In one embodiment, the light chain module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity to one or more of, e.g., one, two, three, or all of, the light chain framework region 1, e.g., LC FR1, light chain framework region 2, e.g., LC FR2, light chain framework region 3, e.g., LC FR3, or light chain framework region 4, e.g., LC FR4. The heavy chain framework regions are depicted in Figures 15B.

In one embodiment, one or more, e.g., one, two, or three, of the CDRs of the heavy chain module differ from the corresponding heavy chain CDRs of an antibody in Table 4 by 1, 2, 3, 4, 5, 6, 7 or more residues.

In one embodiment, one or more, e.g., one, two, or three, of the CDRs of the light chain module differ from the corresponding light chain CDRs of an antibody in Table 4 by 1, 2, 3, 4, 5, 6, 7 or more residues.

In one embodiment, the composition has increased ocular retention when administered, e.g., intravitreally, to a subject compared with the ocular retention of the agent when it is not covalently attached to the OHLE polypeptide, e.g., effective antibody or effective fragment thereof.

In one embodiment, the ocular retention of the composition in the vitreous, aqueous humor, retina, choroid, iris ciliary body, lens, sclera, conjunctiva, and/or cornea is increased. In one embodiment, the retention is increased as indicated by an increase in the half life of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%. In one embodiment, the increase in half life is measured in an assay described herein, e.g., a rabbit pharmacokinetic (PK) model described herein, e.g., in Example 21. In one embodiment, the half life of the composition in the vitreous of a subject is at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 21, 22, 23, 24, or 25 days when the composition is administered intravitreally to the subject.

In another aspect, the invention relates to an engineered agent or composition that includes an effective sequence from an IL-6 antibody described herein (e.g., EBI-029, EBI-030, and/or EBI-031) that improves ocular retention of the agent.

In one embodiment, the OHLE polypeptide binds IL-6, e.g., with an affinity that is at least 50% of that of an antibody from Table 4. In one embodiment, the OHLE polypeptide does not substantially bind to IL-6, e.g., or binds with an affinity that is less than 20%, 10%, or 5% of that of an antibody from Table 4. In some embodiments, the OHLE polypeptide does not bind to IL-6, e.g., no binding to IL-6 can be detected by assays described herein.

Also provided herein are methods of producing engineered agents and methods of using the engineered agents, e.g., in the treatment of eye disease.

In one aspect provided herein is a composition comprising an engineered agent for ocular (e.g., intraocular, e.g., intravitreal) administration. In embodiments, the agent that is engineered, e.g., by being coupled to, e.g., covalently coupled to, an ocular half life extending (OHLE)

polypeptide described herein. In embodiments, the agent that is engineered, e.g., by being couple to, e.g., covalently coupled to, an OHLE described herein, is a therapeutic agent useful for the treatment of an eye disease. In embodiments, the agent comprises a polypeptide.

In embodiments, the agent is a VEGF antagonist, an IL-6 antagonist, an IL-1 antagonist, a complement inhibitor, or a PDGF pathway antagonist, e.g., a PDGF antagonist or a PDGF receptor antagonist; or a functional fragment thereof. Examples of VEGF antagonists include, but are not limited to, aflibercept (Eylea®), bevacizumab (Avastin®), or ranibizumab (Lucentis®). Examples of IL-6 antagonists include, but are not limited to, tocilizumab, sarilumab or siltuximab. Examples of complement inhibitors include, but are not limited to, lampalizumab (anti-Factor D). Examples of a PDGF pathway antagonist e.g., a PDGF antagonist or a PDGFR antagonist includes, but is not limited to, Fovista® or REGN2 176-3.

In embodiments, the agent is aflibercept (Eylea®). In embodiments, the agent is bevacizumab (Avastin®). In embodiments, the agent is lampalizumab (anti-Factor D). In embodiments, the agent is ranibizumab (Lucentis®). In embodiments, the agent is an IL-6 antagonist, e.g., tocilizumab, sarilumab or siltuximab. In embodiments, the agent is an IL-1 antagonist, e.g., arcalyst or canakinumab.

In one embodiment, the agent is coupled to the N terminus of the heavy chain module. In one embodiment, the agent is coupled to the C terminus of the heavy chain module. In one embodiment, the agent is coupled to the N terminus of the light chain module. In one embodiment, the agent is coupled to the C terminus of the light chain module. In one embodiment, two or more agents, e.g., 2, 3, 4, 5, 6, 7, or 8 or more agents are coupled to the OHLE polypeptide.

In embodiments, the agent is an IL-6 antibody or antigen binding fragment thereof and the agent is engineered as described herein. In embodiments, the agent is an antibody or antigen binding fragment described herein, e.g., an antibody or antigen binding fragment comprising a sequence disclosed in Table 4 or an antibody or antigen binding fragment disclosed in WO2014/074905, the entire content of which is hereby incorporated by reference; and the agent is engineered as described herein. In embodiments, the agent is EBI-029, EBI-030, EBI-031 or an antigen binding fragment thereof.

In embodiments, the composition comprises an agent (e.g., a therapeutic agent or a diagnostic agent) coupled, e.g., covalently attached, to an OHLE polypeptide, e.g., an effective

antibody or effective fragment thereof, wherein the composition has increased ocular retention. In this context, and as used elsewhere herein, an "effective antibody," "effective fragment," "effective sequence" and the like, generally refer to an antibody, fragment, or sequence that, when linked with another agent, prolongs the ocular retention of the agent.

In embodiments, the composition has increased ocular retention when administered, e.g., intravitreally, to a subject compared with the ocular retention of the agent when it is not covalently attached to the OHLE polypeptide, e.g., effective antibody or effective fragment thereof.

In embodiments, the composition has increased ocular retention when administered, e.g., intravitreally, to a subject compared with the ocular retention of the agent when it is covalently attached to a control molecule having a molecular weight and/or hydrodynamic radius that is similar to that of the effective antibody or effective fragment thereof. In embodiments, the control molecule is another antibody or antibody fragment that has a similar molecular weight and/or hydrodynamic radius compared with the effective antibody or effective fragment thereof.

In embodiments, the effective antibody comprises or consists of an antibody sequence or fragment disclosed herein, e.g., a sequence disclosed in Table 4, or an effective fragment thereof. In embodiments, the effective antibody or effective fragment comprises or consists of an antibody sequence or fragment disclosed WO2014/074905 the entire content of which is hereby incorporated by reference.

In embodiments, the effective antibody or effective fragment comprises a heavy chain and a light chain. In embodiments, the heavy and light chains are linked by one or more disulfide bonds. In embodiments, the effective antibody or effective fragment is a Fab, Fab', F(ab')2, scFv or Fv fragment. In embodiments, the effective antibody or effective fragment is a Fab. In embodiments, the effective antibody or effective fragment is an scFv.

In some embodiments, the effective antibody or effective fragment binds to an IL-6, e.g., a human IL-6, e.g., to site II of a human IL-6.

In embodiments, the effective antibody or effective fragment inhibits cis-IL-6 signaling, e.g., as assessed based on the IC50 or IC90 value obtained using a HEK-Blue™ assay described herein, e.g., with 20 pM free IL-6. In embodiments, the effective antibody or effective fragment has an IC50 of less than 47 pM and/or an IC90 of less than 4350 pM. In embodiments, the IC50 is less than 47 pM, e.g., less than 40, 30, 20, 10, 5, 4, 3, 2, or 1 pM. In embodiments, the IC90 is

less than 4350 pM, e.g., less than 4000, 2000, 1000, 100, 50, 40, 30, 20, 15, 10, or 5 pM. In embodiments, the IC50 and/or IC90 is assessed in a HEK-Blue™ assay with 20 pM IL-6.

In embodiments, the effective antibody or effective fragment blocks free IL-6 with greater potency compared to tocilizumab, e.g., as assessed based on the IC50 values obtained using a HEK-Blue™ assay with 20 pM IL-6. In embodiments, the effective antibody or effective fragment inhibits IL-6 with more than 900 fold greater potency compared to tocilizumab. In embodiments, the effective antibody or effective fragment is EBI-031 or an effective fragment thereof. In embodiments, the effective antibody or effective fragment has an IC50 of less than 15 pM, e.g., an IC50 of 14.2 pM, for inhibition of IL-6.

In embodiments, the effective antibody or effective fragment blocks trans-IL-6 signaling, e.g., as assessed using a HEK-Blue™ assay described herein, e.g., with 200 pM hyper IL-6. In embodiments, the effective antibody or effective fragment inhibits signaling by hyper IL-6. In embodiments, the effective antibody or effective fragment inhibits signaling by hyper IL-6 with greater potency than tocilizumab, e.g., with more than 900 fold greater potency compared to tocilizumab. In embodiments, the effective antibody or effective fragment inhibits signaling by hyper IL-6 with an IC50 of less than 1 μM. In embodiments, the effective antibody or effective fragment inhibits signaling by hyper IL-6 with an IC50 of less than 1 nM. In embodiments, the effective antibody or effective fragment inhibits signaling by hyper IL-6 with an IC50 of less than 100 pM or less than 50 pM, e.g., with an IC50 of about 14-15 pM. In embodiments, the effective antibody or effective fragment is EBI-031 or an effective fragment thereof.

In embodiments, the effective antibody or effective fragment inhibits cis-IL-6 signaling and trans-IL-6 signaling.

In embodiments, the effective antibody or effective fragment is modified, e.g., by introducing one or more mutations. In some embodiments, the modification (i) decreases or ablates binding to IL-6 and /or (ii) reduces or results in loss of potency of the effective antibody or effective fragment in inhibiting IL-6 activity, e.g., as assessed using one or more assays described herein (e.g., the HEK-Blue assay or T1165 proliferation assay).

Table 4: Summary overview of sequences of EBI-029, EBI-030, and EBI-03 1

Description	SEQ ID NO:	Sequence
EBI-029 HC (IgG2) aa sequence	SEQ ID NO:**11**	QVQLVQSGAE VKKPGSSVKV SCKASGYALS NYLIEWVRQA PGQGLEWMGV ITPGSGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
EBI-029 HC –H311A	SEQ ID NO:**10**	QVQLVQSGAE VKKPGSSVKV SCKASGYALS NYLIEWVRQA PGQGLEWMGV ITPGSGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV **A**QDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
EBI-029 LC aa sequence	SEQ ID NO:**12**	DIVMTQSPDS LAVSLGERAT INCRASESVD NYGIPFMNWY QQKPGQPPKL LIYAASNRGS GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSEEVPL TFGQGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
EBI-029 (IgG1) Fab HC aa sequence	SEQ ID NO:**24**	QVQLVQSGAE VKKPGSSVKV SCKASGYALS NYLIEWVRQA PGQGLEWMGV ITPGSGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPSSKST SGGTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT QTYICNVNHK PSNTKVDKKV EPKSCDKTHT
EBI-029 VH aa sequence	SEQ ID NO:**17**	QVQLVQSGAEVKKPGSSVKVSCKASGYA**L**SNYLIE WVRQAPGQGLEWMGVITPGSGTINYAQKFQGRVTIT ADESTSTAYMELSSLRSEDTAVYYCARSRWDPLYYYALEY WGQGTTVTVSS
EBI-029 VL aa sequence	SEQ ID NO:**18**	DIVMTQSPDSLAVSLGERATINCRASESVDNYGIPFMNWYQQ KPGQPPKLLIYAASNRGSGVPDRFSGSGSGTDFTLTISSLQAE DVAVYYCQQSEEVPLTFGQGTKLEIKRTV
EBI-029 VH CDR1	SEQ ID NO:**4**	GYALSNYLIE
EBI-029 VH CDR2	SEQ ID NO:**5**	VITPGSGTIN
EBI-029 VH CDR3	SEQ ID NO:**6**	SRWDPLYYYALEY
EBI-029 VL CDR1	SEQ ID NO:**7**	RASESVDNYGIPFMN
EBI-029 VL CDR2	SEQ ID NO:**8**	AASNRGS
EBI-029 VL CDR3	SEQ ID NO:**9**	QQSEEVPLT

Description	SEQ ID NO:	Sequence
EBI-030 HC (lgG2) aa sequence	SEQ ID **NO:41**	QVQLVQSGAE VKKPGSSVKV SCKASGYVLP NYLIEWVRQA PGQGLEWMGVTTPGGGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSRWDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVWDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
EBI-030 LC aa sequence	SEQ ID **NO:42**	DIVMTQSPDS LAVSLGERAT INCRASESVD NYGIPFMNWY QQKPGQPPKL LIYAASNRGS GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSEEVPLTFGQGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
EBI-030 (lgG1) Fab HC aa sequence	SEQ ID **NO:39**	QVQLVQSGAE VKKPGSSVKV SCKASGYVLP NYLIEWVRQA PGQGLEWMGVTTPGGGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPSSKST SGGTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT QTYICNVNHK PSNTKVDKKV EPKSCDKTHT
EBI-030 (lgG2) Fab HC aa sequence	SEQ ID **NO:68**	QVQLVQSGAEVKKPGSSVKVSCKASGYVLPNYLIEWVRQAPGQGLEWMGV TTPGGGTINYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSRWD PLYYYALEYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSWTVPSSNFGTQTYTCN VDHKPSNTKVDKTVERK
EBI-030 VH aa sequence	SEQ ID **NO:37**	QVQLVQSGAE VKKPGSSVKV SCKASGYVLP NYLIEWVRQA PGQGLEWMGV TTPGGGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV SS
EBI-030 VL aa sequence	SEQ ID **NO:38**	DIVMTQSPDSLAVSLGERATINCRASESVDNYGIPFMNWYQQKPGQPPKLLIY AASNRGSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSEEVPLTFG QGTKLEIKRTV
EBI-030 VH CDR1	SEQ ID **NO:31**	GYVLPNYLIE
EBI-030 VH CDR2	SEQ ID **NO:32**	VTTPGGGTIN
EBI-030- VH CDR3	SEQ ID **NO:33**	SRWDPLYYYALEY
EBI-030 VL CDR1	SEQ ID **NO:34**	RASESVDNYGIPFMN
EBI-030 VL CDR2	SEQ ID **NO:35**	AASNRGS
EBI-030 VL CDR3	SEQ ID **NO:36**	QQSEEVPLT

Description	SEQ ID NO:	Sequence
EBI-03 1 lgG2 HC aa sequence	SEQ ID NO:47	QVQLVQSGAE VKKPGSSVKV SCKASGYVLP NYLIEWVRQA PGQGLEWMGV TTPGGGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVWDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV AQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
scFv VH-VL aa sequence	SEQ ID NO:65	QVQLVQSGAEVKKPGSSVKVSCKASGYVLPNYLIEWVRQAPGQGLEWMGV TTPGGGTINYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSRWD PLYYYALEYWGQGTTVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSL GERATINCRASESVDNYGIPFMNWYQQKPGQPPKLLIYAASNRGSGVPDRFS GSGSGTDFTLTISSLQAEDVAVYYCQQSEEVPLTFGQGTKLEIKRTV
scFv VL-VH aa sequence	SEQ ID NO:72	DIVMTQSPDSLAVSLGERATINCRASESVDNYGIPFMNWYQQKPGQPPKLLIY AASNRGSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSEEVPLTFGQ GTKLEIKRTVGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCKAS GYVLPNYLIEWVRQAPGQGLEWMGVTTPGGGTINYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCARSRWDPLYYYALEYWGQGTTVTVSS
030 lgG2 constant region	SEQ ID NO: 73	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSV FLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVM HEALHN HYTQKSLSLSPGK
031 lgG2 constant region	SEQ ID NO: 74	ASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVWDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV AQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK

aa= amino acid; HC=heavy chain; LC=light chain; VH=heavy chain variable region; VL=light chain variable region

In embodiments, the effective antibody or effective fragment comprises at least 10 amino acids of a sequence disclosed in Table 4. In embodiments, the effective antibody or effective fragment comprises or consists of at least 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids of an antibody sequence disclosed herein, e.g., a sequence disclosed in Table 4.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises or consists of a sequence that is at least 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95,

96, 97, 98, or 99% identical to an antibody sequence disclosed herein, e.g., a sequence disclosed in Table 4.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises or consists of a sequence that differs by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids from an antibody sequence disclosed herein, e.g., a sequence disclosed in Table 4.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises or consists of a sequence of at least 60, 70, 80, 90, 100, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, or 440 consecutive amino acids of an antibody sequence disclosed herein, e.g., a sequence disclosed in Table 4.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises or consists of a sequence that is at least 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to a sequence of at least 60, 70, 80, 90, 100, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, or 440 consecutive amino acids from an antibody sequence disclosed herein, e.g., a sequence disclosed in Table 4.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises at least one CDR (e.g., a VH CDRl, VH CDR2, VH CDR3, VL CDRl, VL CDR2, and/or VL CDR3) of a sequence disclosed in Table 4. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment comprises at least a VH CDRl, a VH CDR2, and a VH CDR3. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises at least a VL CDRl, a VL CDR2, and a VL CDR3.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a heavy chain sequence comprising a VH CDRl, a VH CDR2, and a VH CDR3. In embodiments, the VH CDRl comprises or consists of SEQ ID NO:4 or SEQ ID NO:3 1. In embodiments, the VH CDR2 comprises or consists of SEQ ID NO:5 or SEQ ID NO:32. In embodiments, the VH CDR3 comprises or consists of SEQ ID NO:6 or SEQ ID NO:22.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises (i) a VH CDRl comprising the sequence of GYXiLX_2NYLIE (SEQ ID NO:45), (ii) a VH CDR2 comprising the sequence of VX_3TPGX_4GTIN (SEQ ID NO:46), and/or (iii) a VH CDR3 comprising the sequence of SRWDPLYYYALEY (SEQ ID NO:6 or SEQ ID NO:33). In embodiments, the effective antibody or effective fragment further comprises (i) a VL CDRl comprising the sequence of SEQ ID NO:7 or SEQ ID NO:34, (ii) a VL CDR2 comprising the

sequence of SEQ ID NO:8 or SEQ ID NO:35, and/or (iii) a VL CDR3 comprising the sequence of SEQ ID NO:9 or SEQ ID NO:36.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a heavy chain variable region comprising a VH CDRl, VH CDR2, and a VH CDR3, wherein (i) the VH CDRl comprises the sequence of GYXiLX$_2$NYLIE (SEQ ID NO:45), (ii) theVH CDR2 comprises the sequence of VX$_3$TPGX$_4$GTIN (SEQ ID NO:46), and/or (ii) the VH CDR3 comprises the sequence of SRWDPLYYYALEY (SEQ ID NO:6 or SEQ ID NO:33).

In embodiments, Xi is A or V, or a conservative substitution for A or V. In embodiments, X_2 is S or P, or a conservative substitution for S or P. In embodiments, X_3 is I or T, or a conservative substitution for I or T. In embodiments, X_4 is S or G, or a conservative substitution for S or G.

In embodiments, Xi is V or a conservative substitution for V. In embodiments, X_2 is P or a conservative substitution for P. In embodiments, X_3 is T or a conservative substitution for T. In embodiments, X_4 is G or a conservative substitution for G. In embodiments, one, two, three or all of the following is true: Xi is V or a conservative substitution for V, X_2 is P or a conservative substitution for P, X_3 is T or a conservative substitution for T, and X_4 is G or a conservative substitution for G. In embodiments, Xi is V or a conservative substitution for V, X_2 is P or a conservative substitution for P, X_3 is T or a conservative substitution for T, and X_4 is G or a conservative substitution for G.

In embodiments, Xi is selected from V, I, L and M. In embodiments, Xi is selected from V, I and L. In embodiments, X_2 is selected from P, G, and A. In embodiments, X_2 is selected from P and G. In embodiments, X_3 is selected from T and S. In embodiments, X_4 is selected from G and P.

In embodiments, one or more (e.g., 1, 2, 3, or all) of the following is true: Xi is V, X_2 is P, X_3 is T, and X_4 is G. In embodiments, Xi is V, X_2 is P, X_3 is T, and X_4 is G.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises (i) a VL CDRl comprising the sequence of SEQ ID NO:7 or SEQ ID NO:34, (ii) a VL CDR2 comprising the sequence of SEQ ID NO:8 or SEQ ID NO:35, and/or (iii) a VL CDR3 comprising the sequence of SEQ ID NO:9 or SEQ ID NO:36. In embodiments, the effective antibody or effective fragment comprises a light chain variable region comprising a VL CDRl, VL CDR2, and a VL CDR3, wherein (i) the VL CDRl comprises the sequence of SEQ ID NO:7

or SEQ ID NO:34, (ii) the VL CDR2 comprises the sequence of SEQ ID NO:8 or SEQ ID NO:35, and/or (iii) the VL CDR3 comprises the sequence of SEQ ID NO:9 or SEQ ID NO:36.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises one or more framework regions of a sequence disclosed herein, e.g., a sequence disclosed in Table 4. The framework regions of the EBl-029, EBl-030, and EBl-031 sequences are shown in Fig. 15A and Fig. 15B.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises one or more constant regions from the heavy chain (e.g., a CHI, a CH2, and/or a CH3) of a heavy chain sequence disclosed herein, e.g., a sequence disclosed in Table 4. The CHI, CH2, and CH3 sequences of the EBl-029, EBl-030, and EBl-031 heavy chain sequences are shown in Fig. 15A. In embodiments, the effective antibody or effective fragment comprises a constant region from a light chain sequence disclosed herein. The CK region of the EBl-029, EBl-030, and EBl-031 light chain sequence is shown in Fig. 15B.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises the hinge region sequence of the heavy chain of an antibody disclosed herein (e.g., EBl-029, EBl-030, or EBl-031). The hinge region sequences of EBl-029, EBl-030, and EBl-031 are shown in Fig. 15A.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a heavy chain and a light chain. In embodiments, the heavy and light chains are linked by one or more disulfide bonds. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment is a Fab or scFv.

In embodiments, the effective OHLE polypeptide, e.g., antibody or effective fragment, comprises a heavy chain variable region sequence comprising or consisting of SEQ ID NO: 17 or SEQ ID NO:37. In embodiments, the effective antibody or effective fragment comprises a heavy chain variable region sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO: 17 or SEQ ID NO:37.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a light chain variable region sequence comprising or consisting of SEQ ID NO: 18 or SEQ ID NO:38. In embodiments, the effective antibody or effective fragment comprises a light chain variable region sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%> identical to SEQ ID NO: 18 or SEQ ID NO:38.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a heavy chain variable region sequence comprising or consisting of SEQ ID NO: 17 or SEQ ID NO:37 and a light chain variable region sequence comprising or consisting of SEQ ID NO:18 or SEQ ID NO:38.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a heavy chain variable region sequence that differs by no more than 5, 4, 3, 2, or 1 amino acids from SEQ ID NO: 17 or SEQ ID NO:37. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a light chain variable region sequence that differs by no more than 5, 4, 3, 2, or 1 amino acids from SEQ ID NO: 18 or SEQ ID NO:38.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a heavy chain variable region comprising or consisting of SEQ ID NO: 17 or SEQ ID NO:37and a light chain variable region comprising or consisting of SEQ ID NO: 18 or SEQ ID NO:38. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises the foregoing heavy and light chain variable regions except that it has a mutation, e.g., a total of at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations. In some embodiments, the mutation(s) decrease or ablate binding of the OHLE polypeptide, e.g., effective antibody or effective fragment, to an IL-6 (e.g., human IL-6). In some embodiments, the mutation(s) results in loss of potency of the antibody, e.g., such that the antibody no longer inhibits IL-6 activity or shows less inhibition of IL-6 activity, e.g., as assessed using an assay described herein (e.g., the HEK-Blue assay or Tl 165 proliferation assay). In alternative embodiments, the mutation(s) does not decrease the affinity of the OHLE polypeptide, e.g., effective antibody or effective fragment, for an IL-6 (e.g., human IL-6). In embodiments, the mutation(s) does not decrease the potency of the OHLE polypeptide, e.g., effective antibody or effective fragment, in inhibiting one or more IL-6 activities.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a heavy chain variable region sequence that differs by no more than 5, 4, 3, 2, or 1 amino acids from SEQ ID NO: 17 or SEQ ID NO:37 and a light chain variable region sequence that differs by no more than 5, 4, 3, 2, or 1 amino acids from SEQ ID NO: 18 or SEQ ID NO:38.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a heavy chain sequence comprising or consisting of SEQ ID NO:24, SEQ ID NO:39 or SEQ ID NO:68. In embodiments, the effective antibody or effective fragment comprises a

heavy chain sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO:24,SEQ ID NO:39, or SEQ ID NO:68. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a heavy chain sequence that differs by no more than 5, 4, 3, 2, or 1 amino acids from SEQ ID NO:24,SEQ ID NO:39, or SEQ ID NO:68. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, is a Fab. In embodiments, the Fab further comprises a light chain sequence, e.g., a light chain sequence as described herein. In embodiments, the Fab comprises SEQ ID NO:24 and SEQ ID NO: 12. In embodiments, the Fab comprises SEQ ID NO:39 and SEQ ID NO:42. In embodiments, the Fab comprises SEQ ID NO:68 and SEQ ID NO:42.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a heavy chain sequence comprising or consisting of SEQ ID NO: 11, SEQ ID NO: 10, SEQ ID NO:41, or SEQ ID NO:47. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a heavy chain sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO:l 1, SEQ ID NO: 10, SEQ ID NO:41, or SEQ ID NO:47. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a heavy chain sequence that differs by no more than 5, 4, 3, 2, or 1 amino acids from SEQ ID NO: 11, SEQ ID NO: 10, SEQ ID NO:41, or SEQ ID NO:47.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a light chain sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO: 12 or SEQ ID NO:42.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a light chain sequence that differs by no more than 5, 4, 3, 2, or 1 amino acids from SEQ ID NO: 12 or SEQ ID NO:42.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a light chain sequence comprising SEQ ID NO: 12 or SEQ ID NO:42.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises a light chain sequence comprising SEQ ID NO: 12 or SEQ ID NO:42 or a sequence that differs by no more than 5, 4, 3, 2, or 1 amino acids from SEQ ID NO: 12 or SEQ ID NO:42. In embodiments, the light chain sequence consists of SEQ ID NO: 12 or SEQ ID NO:42.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises

(i) a VH CDRl comprising the sequence of SEQ ID NO:4 or SEQ ID NO:31, a VH CDR2 comprising the sequence of SEQ ID NO:5 or SEQ ID NO:32, and a VH CDR3 comprising the sequence of SEQ ID NO:6 or SEQ ID NO:33 and (ii) a VL CDRl comprising the sequence of SEQ ID NO:7 or SEQ ID NO:34, a VL CDRl comprising the sequence of SEQ ID NO:8 or SEQ ID NO:35, and a VL CDR3 comprising the sequence of SEQ ID NO:9 or SEQ ID NO:36. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises the foregoing CDRs except that it has a mutation, e.g., a total of at most 1, 2, 3, 4, 5, 6, 7, or 8 mutations in all six of the CDRs.

In some embodiments, the mutation(s) decrease or ablate binding of the OHLE polypeptide, e.g., effective antibody or effective fragment, to an IL-6 (e.g., human IL-6). In some embodiments, the mutation(s) result in loss of potency of the antibody, e.g., such that the antibody no longer inhibits IL-6 activity or shows less inhibition of IL-6 activity, e.g., as assessed using an assay described herein (e.g., the HEK-Blue assay or Tl 165 proliferation assay).

In alternative embodiments, the mutation(s) does not decrease the affinity of the effective antibody or effective fragment for an IL-6 (e.g., human IL-6). In embodiments, the mutation(s) does not decrease the potency of the effective antibody or effective fragment in inhibiting one or more IL-6 activities.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, is an scFv. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises or consists of the scFv sequence

QVQLVQSGAEVKKPGSSVKVSCKASGYVLPNYLIEWVRQAPGQGLEWMGVTTPGGGTI
NYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSRWDPLYYYALEYWGQGT
TVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCRASESVDNYGIPFMN
WYQQKPGQPPKLLIYAASNRGSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSEE
VPLTFGQGTKLEIKRTV (SEQ ID NO:65) or

DIVMTQSPDSLAVSLGERATINCRASESVDNYGIPFMNWYQQKPGQPPKLLIYAASNRG
SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSEEVPLTFGQGTKLEIKRTVGGGGS
GGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCKASGYVLPNYLIEWVRQAPGQGLEW

MGVTTPGGGTINYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSRWDPLY YYALEYWGQGTTVTVSS (SEQ ID NO:72). In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment comprises a sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO:65 or SEQ ID NO:72. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment comprises sequence that differs by no more than 5, 4, 3, 2, or 1 amino acids from SEQ ID NO:65 or SEQ ID NO:72. In embodiments, the OHLE polypeptide is not an scFv.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, is an IgGl, an IgG2, an IgG3, or an IgG4 antibody or fragment thereof. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment is an IgGl or an IgG2 antibody or fragment thereof. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, is an IgGl Fab or an IgG2 Fab.

In embodiments, the OHLE polypeptide, e.g., effective or effective fragment, is engineered to reduce or eliminate ADCC activity.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, is an IgG2 antibody or fragment thereof.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, is a monoclonal antibody or fragment thereof. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment is a humanized or human monoclonal antibody or a fragment thereof.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment comprises or consists of an IgG2 sequence or a portion thereof, or a sequence having 60, 70, 80, 90, 95, or 99% identity thereto.

In embodiments, the IgG2 heavy chain sequence is SEQ ID NO:41, or a sequence having 60, 70, 80, 90, 95, or 99% identity thereto.

In embodiments, the IgG2 light chain sequence is SEQ ID NO:42, or a sequence having 60, 70, 80, 90, 95, or 99% identity thereto.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises or consists of a portion of an IgG2 sequence, e.g., the Fc domain, e.g., an Fc domain having the sequence of SEQ ID NO:51, or a sequence having 60, 70, 80, 90, 95, or 99% identity thereto.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises or consists of a portion of an IgG2 sequence, e.g., the CH2 domain, e.g., a CH2 domain having the sequence
VECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTK
(SEQ ID NO:66), or a sequence having 60, 70, 80, 90, 95, or 99% identity thereto.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment comprises or consists of a portion of an IgG2 sequence, e.g., the CH3 domain, e.g., a CH3 domain having the sequence
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:67),
or a sequence having 60, 70, 80, 90, 95, or 99% identity thereto.

In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises or consists of a portion of an IgG2 sequence, e.g., an IgG2 Fab fragment. In embodiments, the OHLE polypeptide, e.g., effective antibody or effective fragment, comprises the following IgG2 Fab heavy chain sequence
QVQLVQSGAEVKKPGSSVKVSCKASGYVLPNYLIEWVRQAPGQGLEWMGVTTPGGGTI
NYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSRWDPLYYYALEYWGQGT
TVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERK
(SEQ ID NO:68), , or a sequence having 60, 70, 80, 90, 95, or 99% identity thereto
and optionally the light chain sequence of SEQ ID NO:42, , or a sequence having 60, 70, 80, 90, 95, or 99% identity thereto.

In embodiments, the retention of the composition in the eye, e.g., in the vitreous, aqueous humor, retina, choroid, conjunctiva, cornea, lens, iris ciliary body, and/or sclera, is increased. In embodiments, the retention is increased as indicated by an increase in the half life of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%. In embodiments, the half life of the composition, e.g., in the vitreous, is increased by at least two fold.

In embodiments, the half life of the composition in the vitreous of a subject is at least 8, 9, or 10 days when the composition is administered intraocularly, e.g., intravitreally, to the subject. In embodiments, the subject is a non-human animal, e.g., a rabbit.

In embodiments, the half life of the composition in the vitreous of a subject is at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 21, 22, 23, 24, or 25 days when the composition is administered intraocularly, e.g., intravitreally, to the subject.

In embodiments, the composition is retained in the eye for at least 1 month, 2 months, 3 months, 4 months, 5 months, or 6 months, e.g., following intravitreal administration.

In embodiments, the subject is a human.

The agent can be covalently attached to the OHLE polypeptide, e.g., effective antibody or effective fragment thereof by any means known in the art, e.g., using chemical methods or by creating a fusion protein using genetic methods.

Chemical methods for attaching an agent and an OHLE polypeptide, e.g., effective antibody or effective fragment thereof include covalent attachment of the agent to the side chains of the amino acid residues of the effective antibody or effective fragment, for example amine, carboxyl, phenyl, thiol or hydroxyl groups. Various conventional coupling agents may be used for this purpose, for example, diisocyanates, diisothiocyanates, bis(hydroxysuccinimide) esters, carbodiimides, maleimide-hydroxysuccinimide esters, glutaraldehyde and the like.

In some embodiments, the linker comprises a non-natural amino acid. Examples of naturally-occurring amino acids that are not naturally-encoded include, but are not limited to, N-acetylglucosaminyl-L-serine, N-acetylglucosaminyl-L-threonine, and O-phosphotyrosine, and other amino acids which do not occur naturally and may be obtained synthetically or may be obtained by modification of non-natural amino acids. In some embodiments, introduction of at least one non-natural amino acid into a polypeptide (e.g., an effective antibody or effective fragment thereof) allows for the application of chemical conjugation to agents via specific chemical reactions with one or more non-natural amino acids while not reacting with the commonly occurring 20 amino acids as described, for example, in U.S. Patent Nos. 7,638,491, 7,632,823, and 8,791,231.

In embodiments, the agent is attached to an OHLE polypeptide, e.g., an effective anti-IL-6 antibody or effective fragment thereof via a linker.

In embodiments, the linker is non-peptide linker, e.g., a PEG linker. In embodiments, the nonpeptide linker includes a reactive group, e.g., a maleimide, on each end.

In embodiments, the linker is a peptide linker.

In embodiments, the peptide linker comprises one or more of the sequences (G4S)x (SEQ ID NO: 75), (GS)x, (GSS)x, (GGGS)x (SEQ ID NO: 76), (GGGGS)x (SEQ ID NO: 77), (GGSG)x (SEQ ID NO: 78), GSAR (SEQ ID NO: 79), AS, and SS; e.g., wherein x is, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In embodiments, the peptide linker is selected from (G4S)x (SEQ ID NO: 75), (GS)x, (GSS)x, (GGGS)x (SEQ ID NO: 76), (GGGGS)x (SEQ ID NO: 77), (GGSG)x (SEQ ID NO: 78), GSAR (SEQ ID NO: 79), AS, and SS; e.g., wherein x is, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 .

In another aspect provided herein is a method of treatment comprising administering a composition disclosed herein to a subject having an ocular disease, thereby treating the subject.

In embodiments, a composition disclosed herein is for use in the treatment of a subject having an ocular disease.

In embodiments, a composition disclosed herein is for use in the preparation of a medicament for the treatment of an ocular disease.

In another aspect provided herein is a method of producing a composition for ocular (e.g., intravitreal) administration, the method comprising covalently attaching an agent to OHLE polypeptide, e.g., an effective antibody or effective fragment thereof. In embodiments, the method further comprises formulating the composition for ocular (e.g., intravitreal) administration. In embodiments, the composition has increased ocular retention when administered, e.g., intravitreally, to a subject compared with the ocular retention of the agent when it is not covalently attached to the effective antibody or effective fragment thereof. In embodiments, the composition has increased ocular retention when administered, e.g., intravitreally, to a subject compared with the ocular retention of the agent when the agent is covalently attached to a control molecule having a molecular weight and/or a hydrodynamic radius that is similar to that of the effective antibody or effective fragment thereof.

In embodiments, the retention of the agent in the vitreous, aqueous humor, retina, conjunctiva, cornea, iris ciliary body, lens, sclera, and/or choroid is increased.

In embodiments, the composition, when intravitreally administered, permeates to one or more other compartments of the eye other than the vitreous. For example, the composition, when intravitreally administered, permeates the aqueous humor, the retina, the conjunctiva, the cornea, the iris ciliary body, the lens, the sclera, and/or the choroid. Permeation to other compartments of the eye is determined by presence of the composition in that compartment or compartments.

In embodiments, the composition is retained in the eye (e.g., in the vitreous, aqueous humor, retina, conjunctiva, cornea, iris ciliary body, lens, sclera, and/or choroid) for at least 1 month, 2 months, 3 months, 4 months, 5 months, or 6 months, e.g., following intravitreal administration.

In embodiments, the retention is increased as indicated by an increase in the half life of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%. In embodiments, the half life of the composition, e.g., in the vitreous, is increased by at least two fold.

In embodiments, the half life of the composition in the vitreous of a subject is at least 8, 9, or 10 days when the composition is administered intraocularly, e.g., intravitreally, to the subject. In embodiments, the subject is a non-human animal, e.g., a rabbit.

In embodiments, the half life of the composition in the vitreous of a subject is at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 21, 22, 23, 24, or 25 days when the composition is administered intraocularly, e.g., intravitreally, to the subject. In embodiments, the subject is a human.

In embodiments, the agent is attached to an OHLE polypeptide, e.g., an effective anti-IL-6 antibody or effective fragment thereof via a linker.

In embodiments, the linker is a PEG linker.

In embodiments, the linker is a peptide linker.

In embodiments, the peptide linker comprises one or more of the sequences (G4S)x (SEQ ID NO: 75), (GS)x, (GSS)x, (GGGS)x (SEQ ID NO: 76), (GGGGS)x (SEQ ID NO: 77), (GGSG)x (SEQ ID NO: 78), GSAR (SEQ ID NO: 79), AS, and SS; wherein x is, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In embodiments, the peptide linker is selected from (G4S)x (SEQ ID NO: 75), (GS)x, (GSS)x, (GGGS)x (SEQ ID NO: 76), (GGGGS)x (SEQ ID NO: 77), (GGSG)x (SEQ ID NO: 78), GSAR (SEQ ID NO: 79), AS, and SS; wherein x is, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

Also provided herein are methods of making an OHLE polypeptide comprising, wherein the method includes providing an antibody; altering, e.g., mutating, the sequence of the antibody such that is more similar to the sequence of an antibody in Table 4, thereby making an OHLE polypeptide. In an embodiment, the antibody is altered, e.g., mutated, such that the antibody comprises at least 60, 70, 75, 80, 85, 90, 95, or 99%> identical to the sequence of the heavy variable region, the IgG2 constant region, or the framework region of an antibody in Table 4. In

an embodiment, the antibody is altered, e.g., mutated, such that the antibody comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identical to the sequence of the light variable region of an antibody in Table 4.

Also provided herein are methods for making an improved therapeutic or diagnostic agent that has increased ocular retention, wherein comprising coupling, e.g., by making a fusion protein, an OHLE polypeptide to a therapeutic or diagnostic agent. Methods for making a fusion protein are known in the art.

In embodiments, the retention of the improved therapeutic or diagnostic agent compared with that of the unaltered agent is increased as indicated by an increase in the half life of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, e.g., as determined by the rabbit PK model described herein. In embodiments, the half life of the improved therapeutic or diagnostic agent, e.g., in the vitreous, aqueous humor, retina, conjunctiva, cornea, iris ciliary body, lens, sclera, and/or choroid, is increased by at least two fold.

In embodiments, the half life of the improved therapeutic or diagnostic agent in the vitreous, aqueous humor, retina, conjunctiva, cornea, iris ciliary body, lens, sclera, and/or choroid of a subject is at least 8, 9, or 10 days when the improved therapeutic or diagnostic agent is administered intravitreally to the subject.

In embodiments, the half life of the improved therapeutic or diagnostic agent in the vitreous, aqueous humor, retina, conjunctiva, cornea, iris ciliary body, lens, sclera, and/or choroid ofa subject is at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 21, 22, 23, 24, or 25 days when the improved therapeutic or diagnostic agent is administered intraocularly, e.g., intravitreally, to the subject.

In embodiments, the improved therapeutic or diagnostic agent is retained in the eye (e.g., in the vitreous, aqueous humor, retina, and/or choroid) for at least 1 month, 2 months, 3 months, 4 months, 5 months, or 6 months following ocular administration, e.g., following intravitreal administration.

In embodiments, the subject is a human.

In embodiments, the composition is for use in the preparation of a medicament for the treatment of an ocular disease described herein, e.g., an IL-6 mediated ocular disease.

In embodiments, the composition is for use in treating an ocular disease described herein, e.g., an IL-6 mediated ocular disease.

Also provided herein is a method of treatment comprising administering the composition to a subject having an ocular disease, thereby treating the subject.

Also provided herein is a container or drug delivery device containing a composition or an engineered agent described herein. In embodiments, the device is configured for delivery of a compound to the vitreous.

Also provided herein is a kit comprising a composition or an engineered agent disclosed herein and optionally, instructions for use.

The entire disclosure of each patent document and scientific article referred to herein, and those patent documents and scientific articles cited thereby, is expressly incorporated by reference herein for all purposes.

Additional features and advantages of the invention are more particularly described below.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph illustrating results of an experiment in which an anti-IL-6 antibody was administered IVT in rat CNV model. Anti-VEGF antibody was administered as a positive control and the negative control was vehicle alone. p = 0.0054 on Day 15 and p = 0.0005 on Day 22 for anti-IL-6 vs. vehicle control.

Fig. 2 is a graph illustrating results of a binding experiment testing the ability of the murine 64 antibody to inhibit binding of IL-6/IL-6R to gpl30.

Fig 3A is a graph illustrating an experiment in which 020 was tested for the ability to block IL-6 signaling in the absence of an excess of soluble IL-6Ra. Experiments were performed in HEK-Blue-IL-6 cells with 0.2 ng/niL IL-6 and 2µg/mL IL6Ra.

Fig 3B is a graph illustrating an experiment in which 020 was tested for the ability to block IL-6 signaling in the presence of an excess of soluble IL-6Ra. Experiments were performed in HEK-Blue-IL-6 cells with 0.2 ng/niL IL-6 and 2µg/mL IL6Ra.

Fig. 4 is a graph illustrating the results of an experiment in which a monoclonal anti-IL-6 antibody ("IL-6 Blockade") was administered IVT in a mouse CNV model. Controls were no treatment (contralateral eye), intravitreal injection of an anti-VEGF antibody ("VEGF

Blockade") or intravitreal injection of an anti-HRP isotype control antibody ("Control Antibody").

Fig. 5 shows the binding to IL-6, relative to the wild type antibody (EBI-029), in antibodies having the following mutations (1) I51T/S55G, (2) A28V/I51T/S55G, (3) S30P/I51T,/S55G, and (4) A28V/S30P/I51T/S55G (also referred to as EBI-030).

Fig. 6 shows the fractional signaling in HEK-Blue™ IL6 reporter cells treated with IL-6 and one of the following Fabs: (1) WT (EBI-029), (2) A28V/I51T/S55G, (3) S30P/I51T/S55G, (4) A28V/S30P/I51T/S55G (EBI-030).

Fig. 7 shows the luminescence (a measure of IL-6 induced proliferation) in T1 165.85.2.1 cells treated with IL-6 and one of the following Fabs at the concentration shown: (1) WT (EBI-029), (2) A28V/I51T/S55G, (3) S30P/I51T/S55G, (4) A28V/S30P/I51T/S55G (EBI-030).

Fig. 8 shows fractional signaling in HEK-Blue™ IL6 reporter cells treated with 20 pM IL-6 and various concentrations of (1) EBI-029 IgG2 (EBI029) produced in HEK-6E cells, (2) EBI-030 IgG2 (EBI030) produced in HEK-6E cells, and (3) EBI-030 IgG2-H31 1A (EBI030 H31 1A) produced in HEK-6E cells; (4) tocilizumab (TOCI), and (5) EBI-030 IgG2 produced in a stable CHO pool (EBI-030 CHO).

Fig. 9 depicts the pharmacokinetic model described in Example 20.

Fig. 10 depicts the effect of increasing antibody potency on the duration of IL-6 inhibition in the eye, as simulated using the pharmacokinetic model described in Example 20.

Fig. 11 shows the drug concentration of EBI-029, EBI-029-H31 1A, EBI-030, EBI-030-H31 1A, Eylea®, and tocilizumab (TCZ) in the vitreous over time following intravitreal administration.

Fig. 12 shows the drug concentration of EBI-029, EBI-030, EBI-030-H31 1A, Eylea®, and tocilizumab (TCZ) in the retina over time following intravitreal administration.

Fig. 13 shows the drug concentration of EBI-029, EBI-030, EBI-030-H31 1A, Eylea®, and tocilizumab (TCZ) in the aqueous humor over time following intravitreal administration.

Fig. 14 shows the drug concentration of EBI-029, EBI-030, EBI-030-H31 1A, Eylea®, and tocilizumab (TCZ) in the choroid over time following intravitreal administration.

Fig. 15A depicts the locations of FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4, CHI, hinge, CH2, and CH3 in the heavy chain sequences of EBI-029 (SEQ ID NO: 11), EBI-030

(SEQ ID NO: 41), and EBI-031 (EBI-031 is also referred to herein as EBI-030-H31 1A) (SEQ ID NO: 47).

Fig. 15B depicts the locations of FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4, and CK in light chain sequence (EBI-029, EBI-030 and EBI-031 have the same light chain sequence) (SEQ ID NO: 12).

Fig. 16A shows the fractional signaling in HEK-Blue™ IL-6 reporter cells treated with 20 pM IL-6 and various concentrations of EBI-031 or tocilizumab.

Fig. 16B shows the fractional signaling in HEK-Blue™ IL-6 reporter cells treated with 200 pM hyper IL-6 and various concentrations of EBI-031 or tocilizumab.

Fig. 17 shows results of computational simulations described in Example 24.

Fig. 18A is a graph showing the pharmacokinetic data from an African green monkey (K797), as described in Example 26.

Fig. 18B is a graph showing the pharmacokinetic data from an African green monkey (K679), as described in Example 26.

Fig. 19 is a graph showing the pharmacokinetic data from both African green monkeys (K797 or K679) and fit curves.

Fig. 20A shows the drug concentration of EBI-031 in the vitreous humor over time following intravitreal administration.

Fig. 20B shows the drug concentration of EBI-031 in the aqueous humor over time following intravitreal administration.

Fig. 20C shows the drug concentration of EBI-031 in the choroid over time following intravitreal administration.

Fig. 20D shows the drug concentration of EBI-031 in the conjunctiva over time following intravitreal administration.

Fig. 20E shows the drug concentration of EBI-031 in the cornea over time following intravitreal administration.

Fig. 20F shows the drug concentration of EBI-031 in the iris ciliary body over time following intravitreal administration.

Fig. 20G shows the drug concentration of EBI-031 in the lens over time following intravitreal administration.

Fig. 20H shows the drug concentration of EBI-031 in the retina over time following intravitreal administration.

Fig. 201 shows the drug concentration of EBI-031 in the sclera over time following intravitreal administration.

Fig. 21A is a graph showing the first analysis of protein concentration in the vitreous of rabbit eyes for each tested protein, EBI-030 IgGl, EBI-030 IgG2, TCZ IgGl, TCZ IgG2, and TCZ X3 IgGl.

Fig. 21B is a graph showing the second analysis of protein concentration in the vitreous of rabbit eyes for each tested protein, EBI-030 IgGl, EBI-030 IgG2, TCZ IgGl, TCZ IgG2, and TCZ X3 IgGl.

Fig. 22 is a graph showing the protein concentration in the retina of rabbit eyes for each tested protein, EBI-030 IgGl, EBI-030 IgG2, TCZ IgGl, TCZ IgG2, and TCZ X3 IgGl.

DETAILED DESCRIPTION

As used herein, singular terms, including but not limited to "a," "an", or "the," include the plural, unless the context clearly indicates otherwise.

As used herein, an "ocular half life extending polypeptide" or "OHLE polypeptide", refers to a polypeptide that comprises a heavy chain module, e.g., an immunoglobulin heavy chain, comprising a heavy chain variable region module and an IgG2 module; and a light chain module, e.g., an immunoglobulin light chain, comprising a light chain variable region module. In an embodiment, a heavy chain variable region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% sequence identity with a heavy chain variable region of an antibody in Table 4. In an embodiment, an IgG2 constant region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% sequence identity with one or more domains, e.g., CHI, CH2 or CH3, of an IgG2 constant region, e.g., SEQ ID NO: 73 or 74. In an embodiment, a light chain variable region module comprises at least at least 60, 70, 75, 80, 85, 90, 95, or 99%> sequence identity with a light chain variable region of an antibody in Table 4.

The OHLE polypeptide results in increased ocular retention, e.g., as measured by determination of half-life as described herein, of an agent coupled thereto. In an embodiment, the ocular retention of the agent, as compared to an otherwise similar agent not coupled to the OHLE polypeptide is increased by 1, 2, 3, 4, 5, 6, 7, or more days, e.g., as evaluated in a rabbit

PK assay described in Example 21. In some embodiments, the ocular retention of the agent is at least 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. In other embodiments, the OHLE polypeptide itself has increased ocular retention, e.g., e.g., as measured by determination of half-life as described herein e.g., as evaluated in a rabbit PK assay described in Example 21.

As used herein, the term "antibody" is synonymous with immunoglobulin and is to be understood as commonly known in the art. The term antibody is not limited by any particular method of producing the antibody. For example, the term antibody includes, inter alia, recombinant antibodies, monoclonal antibodies, and polyclonal antibodies. As used herein, an antibody is a tetramer, and unless otherwise disclosed, each is composed of two identical pairs of polypeptide chains, each pair having one light chain and one heavy chain. The amino terminus of each chain comprises a variable region of about 100 to 120 or more amino acids that play a primary role in antigen recognition. The carboxy-terminal portion of each chain comprises a constant region with a primary role in antibody effector function. Classes of human light chains are termed kappa and lambda light chains. Heavy chain classes are mu, delta, gamma, alpha, or epsilon, and define the isotype of an antibody. Antibody isotypes are IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about three or more amino acids.

The variable regions of each heavy/light chain pair (VH and VL), respectively, form the antigen binding site. Accordingly, an intact IgG antibody, for example, has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

Variable regions of antibody heavy and light chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also termed complementary determining regions or CDRs. The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are involved in the binding and specificity of each particular antibody for its particular antigen. Variability lies primarily in the CDRs, which are separated by the more highly conserved framework regions (FRs). The assignment of amino acids to each domain is made in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia and Lesk, J Mol Biol 196:901-917 (1987); Chothia et al, Nature 342:878-883 (1989), which describe methods known in the art.

"Wild type" can refer to the most prevalent allele or species found in a population or to the antibody obtained from a non-manipulated animal, as compared to an allele or polymorphism, or a variant or derivative obtained by a form of manipulation, such as mutagenesis, use of recombinant methods and so on to change an amino acid of the antigen-binding molecule.

The term "antibody fragment" refers to a portion of an intact or a full-length chain or an antibody, generally the target binding or variable region. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, scFv and Fv fragments. A "functional fragment" or "analog of an anti-IL-6 site II antibody" is a fragment that can prevent or substantially reduce the ability of IL-6 to bind to a receptor, reduce the ability of IL-6/IL-6R complex to bind to gpl30, or reduce the ability of ligand to bind to gpl30 or to initiate signaling. As used herein, "an antigen binding fragment" or "functional fragment" can refer to fragments, such as Fv, Fab, F(ab')2 and so on which can prevent or substantially reduce the ability of IL-6 to bind to a receptor, reduce the ability of IL-6/IL-6R complex to bind to gpl30, or to initiate signaling.

The term "percent sequence identity" in the context of nucleic acid sequences means the residues in two sequences that are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over at least about nine nucleotides, for example, at least about 18 nucleotides, at least about 24 nucleotides, at least about 28 nucleotides, at least about 32 nucleotides, at least about 36 nucleotides, or at least about 48 or more nucleotides. Algorithms known in the art can be used to measure nucleotide sequence identity. For example, polynucleotide sequences can be compared using FASTA, Gap or Bestfit (Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, WI). FASTA, includes, e.g., the programs FASTA2 and FASTA3, provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, Methods Enzymol 183:63-98 (1990); Pearson, Methods Mol Biol 132:185-219 (2000); Pearson, Methods Enzymol 266:227-258 (1996); Pearson, J Mol Biol 276:71-84 (1998); incorporated herein by reference). Default parameters for a particular program or algorithm are typically used. For example, percent sequence identity between nucleic acid sequences can be determined using FASTA with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) or using Gap with its default parameters as provided in GCG Version 6.1, incorporated herein by reference.

The term "percent sequence identity" in the context of amino acid sequences means the residues in two sequences that are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over at least about five amino acid residues, for example, at least about 20 amino acid residues, at least about 30 amino acid residues, at least about 50 amino acid residues, at least about 100 amino acid residues, at least about 150 amino acid residues, or at least about 200 or more amino acid residues. Sequence identity for polypeptides is typically measured using sequence analysis software. Algorithms for determination of percent sequence identity are known in the art. For example, amino acid sequences can be compared using FASTA, Gap or Bestfit (Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, WI). Protein analysis software matches sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For example, GCG contains programs such as "Gap" and "Bestfit," which can be used with default parameters as specified by the programs to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and an analog thereof. See, e.g., GCG Version 6.1 (University of Wisconsin, Madison, WI). Polypeptide sequences also can be compared using FASTA using default or recommended parameters, see GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, Methods Enzymol 183:63-98 (1990); Pearson, Methods Mol Biol 132:185-219 (2000)). Another algorithm that can be used when comparing a sequence to a database containing a large number of sequences from different organisms is the computer program BLAST, e.g., blastp or tblastn, using default parameters as supplied with the programs. See, e.g., Altschul et al, J Mol Biol 215:403-410 (1990); Altschul et al, Nucleic Acids Res 25:3389-402 (1997).

The term "substantial similarity" when referring to a nucleic acid or fragment thereof, means that when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 85%, at least about 90%, and at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, for example, 85%>, 90%>, 95%>, 96%>, 98%>, or 99%> sequence identity as measured by any known algorithm of sequence identity, such as FASTA, BLAST or Gap.

As applied to polypeptides, the term "substantial identity" or "substantial similarity" means that two amino acid sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights as supplied with the programs, share at least about 70%, 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity; e.g., 70%, 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity. In certain embodiments, residue positions that are not identical differ by conservative amino acid substitutions.

"Therapeutically effective amount" refers to that amount of a therapeutic agent being administered that will ameliorate at least one sign or symptoms a disease being treated or enhance or improve the prophylactic and or therapeutic effect(s) of another therapy (e.g., another therapeutic agent) useful for treating an IL-6 associated disease. It is understood that the therapeutically effective amount may be administered in multiple doses over a limited amount of time or as a chronic treatment.

"Treat", "treating" and "treatment" refer to a method of ameliorating a signs or symptoms or a disease.

As used herein, the term "disease" includes diseases and disorders.

Antibody residues can be modified as discussed and exemplified herein e.g., using site-directed mutagenesis. For example, amino acid changes can be made in one or more framework regions and/or one or more CDRs using methods and parameters known in the art.

A change in the amino acid sequence can include substituting one or more amino acid residue(s) with a non-naturally occurring or non-standard amino acid, modifying one or more amino acid residue into a non-naturally occurring or non-standard form, or inserting one or more non-naturally occurring or non-standard amino acids into the sequence. Examples of numbers and locations of alterations in sequences of the invention are described elsewhere herein. Naturally occurring amino acids include the 20 "standard" L-amino acids identified as G, A, V, L, I, M, P, F, W, S, T, N, Q, Y, C, K, R, H, D, E by their standard single-letter codes. Non-standard amino acids include any other residue that may be incorporated into a polypeptide backbone or result from modification of an existing amino acid residue. Non-standard amino acids may be naturally occurring or non-naturally occurring. Several naturally occurring non-standard amino acids are known in the art, such as 4-hydroxyproline, 5-hydroxylysine, 3-methylhistidine, and N-acetylserine. Those amino acid residues that are derivatized at their N-

alpha position will only be located at the N-terminus of an amino-acid sequence. The amino acid is typically an L-amino acid. In some cases the amino acid is a D-amino acid. Alteration may therefore comprise modifying an L-amino acid into, or replacing it with, a D-amino acid. Methylated, acetylated and/or phosphorylated forms of amino acids are also known, and amino acids in the present invention may be subject to such modification. Non-standard amino acids (e.g., D-amino acids) can be incorporated into an amino acid sequence using methods known in the art, for example in synthesis of the molecule or by post-synthesis modification or replacement of an amino acid.

Novel VH or VL regions carrying CDR-derived sequences of the invention may be generated using random mutagenesis of one or more selected VH and/or VL nucleic acid sequences to generate mutations within the entire variable domain. For example, error-prone PCR can be used (Chao et al, Nature Protocols, 1:755-768 (2006)). In some embodiments one or two amino acid substitutions are made within an entire variable domain or set of CDRs. Other methods know in the art can be used to generate mutations, for example site-directed mutagenesis, typically in one or more CDRs.

One method for producing an antibody is to alter a VH domain such as those disclosed herein by adding, deleting, substituting or inserting one or more amino acids. The altered VH domain can be combined with a VL domain (e.g., a VL domain disclosed herein), which can also be altered as described herein and using methods known in the art. In some cases, a variant VH or VL domain can have 1, 2, 3, 4, or 5 such alterations (e.g., 1, 2, 3, 4, or 5 amino acid substitutions).

Antibody fragments can be generated using methods known in the art such as recombinant DNA, enzymatic cleavage (for example, using pepsin or papain), chemical cleavage of an antibody (for example, chemical reduction of disulfide bridges). Antibody fragments that comprise an antibody antigen-binding site include, but are not limited to, molecules such as Fab, Fab', Fab'-SH, scFv, Fv, dAb, Fd, and disulfide stabilized variable region (dsFv). Various other antibody molecules including one or more antibody antigen-binding sites can be engineered, including for example F(ab')2, F(ab)3, diabodies, triabodies, tetrabodies, and minibodies. Examples of antibody molecules and methods for their construction and use are described in Holliger and Hudson, 2005, Nat Biotechnol 23:1 126-1 136. Non-limiting examples of binding fragments are a Fab fragment composed of VL, VH, constant light chain domain (CL) and

constant heavy chain domain 1 (CHI) domains; an Fd fragment composed of VH and CHI domains; an Fv fragment composed of the VL and VH domains of a single antibody; a dAb fragment composed of a VH or a VL domain; isolated CDR regions; an F(ab')2 fragment, a bivalent fragment comprising two linked Fab fragments; a single chain Fv molecule (scFv), in which a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site; a bispecific single chain Fv dimer (for example as disclosed in WO 1993/01 1161) and a diabody, which is a multivalent or multispecific fragment constructed using gene fusion (for example as disclosed in WO94/13804). Fv, scFv, or diabody molecules can be stabilized by the incorporation of disulfide bridges linking the VH and VL domains. Minibodies comprising an scFv joined to a CH3 domain can also be used as an IL-6a. Other fragments and derivatives of an antibody that can be used as an IL-6a include a Fab', which differs from a Fab fragment by the addition of a few amino acid residues at the carboxyl terminus of the heavy chain CHI domain, including one or more cysteines from the antibody hinge region, and Fab'-SH, which is a Fab' fragment in which the cysteine residue(s) of the constant domains bear a free thiol group.

In some cases, an antibody or fragment thereof is modified to improve or introduce a desirable property, for example PEGylation to increase half-life or incorporation.

A dAb (domain antibody) is a small monomeric antigen-binding fragment of an antibody (the variable region of an antibody heavy or light chain). VH dAbs occur naturally in camelids (e.g., camels and llamas) and can be produced by immunizing a camelid with a target antigen, isolating antigen-specific B cells and directly cloning dAb genes from individual B cells. A dAb can comprise a VH or VL domain substantially as set out herein, or a VH or VL domain comprising a set of CDRs substantially as set out herein.

Antibodies of the invention include bispecific antibodies in which two different variable regions are combined in the same molecule. For example, a bispecific antibody can be prepared chemically or from hybrid hybridomas. Such a molecule can be a bispecific antibody fragment of a type discussed above. One non-limiting example of a method for generating a bispecific antibody is BiTE™ technology in which the binding domains of two antibodies with different specificity can be used and directly linked via short flexible peptides. This combines two antibodies on a short single polypeptide chain. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction.

Bispecific antibodies can be constructed as entire IgG, as bispecific Fab'2, as Fab'PEG, as diabodies or else as bispecific scFv. Further, two bispecific antibodies can be linked using routine methods known in the art to form tetravalent antibodies.

Bispecific diabodies, as opposed to bispecific whole antibodies, are useful, in part because they can be constructed and expressed in *E. coli.* Diabodies (and many other polypeptides, such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO 1994/13804) from libraries. If one arm of the diabody is to be kept constant, for example, with a specificity directed against site II of IL-6, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected.

Bispecific whole antibodies may be made by alternative engineering methods as described in described in WO 1996/2701 1, WO 1998/50431 and WO 2006/028936.

In some cases, a therapeutic agent comprises an antigen-binding site within a non-antibody molecule, for example, by incorporating one or more CDRs, e.g. a set of CDRs, in a non-antibody protein scaffold, as discussed further below. In some cases, the CDRs are incorporated into a non-antibody scaffold. An target binding binding site can be provided by an arrangement of CDRs on non-antibody protein scaffolds, such as fibronectin or cytochrome B, or by randomizing or mutating amino acid residues of a loop within a protein scaffold to confer binding specificity for a target. Scaffolds for engineering novel binding sites in proteins are known in the art. For example, protein scaffolds for antibody mimics are disclosed in WO200034784, which describes proteins (antibody mimics) that include a fibronectin type III domain having at least one randomized loop. A suitable scaffold into which to graft one or more CDRs, e.g., a set of HCDRs, can be provided by any domain member of the immunoglobulin gene superfamily. The scaffold can be a human or non-human protein. An advantage of a non-antibody protein scaffold is that it can provide an antigen-binding site in a scaffold molecule that is smaller and/or easier to manufacture than at least some antibody molecules. Small size of a binding member may confer useful physiological properties, such as an ability to enter cells, penetrate deep into tissues or reach targets within other structures, or to bind within protein cavities of the target antigen. Typical are proteins having a stable backbone and one or more variable loops, in which the amino acid sequence of the loop or loops is specifically or randomly mutated to create an antigen-binding site that binds the target antigen. Such proteins include the IgG-binding domains of protein A from *S. aureus,* transferrin, tetranectin, fibronectin (e.g., using

the 10th fibronectin type III domain), lipocalins as well as gamma-crystalline and other Affilin™ scaffolds (Scil Proteins, Halle, Germany). Examples of other approaches include synthetic microbodies based on cyclotides—small proteins having intra-molecular disulfide bonds, microproteins (e.g., Versabodies™, Amunix Inc., Mountain View, CA) and ankyrin repeat proteins (DARPins, e.g., from Molecular Partners AG, Zurich-Schlieren, Switzerland). Such proteins also include small, engineered protein domains such as, for example, immuno-domains (see for example, U.S. Patent Publication Nos. 2003/082630 and 2003/157561). Immuno-domains contain at least one complementarity determining region (CDR) of an antibody.

Agents

Agents that can be engineered as described herein (e.g., by covalently attaching an ocular half-life extending (OHLE) polypeptide, e.g., an effective antibody or effective fragment) include therapeutic and/or diagnostic agents for ocular administration. Typically, the agents are for intraocular, e.g., intravitreal, administration. In embodiments, the engineered agent or composition comprising the engineered agent has improved pharmacokinetic properties. In embodiments, the engineered agent has improved retention in the eye compared with the agent. Retention in eye can be assessed using standard pharmacokinetic parameters, such as, e.g., half life and clearance. Longer half life and/or reduced clearance in the eye, e.g., in the vitreous of the eye, indicates improved retention.

In embodiments, the agent is selected from lampalizumab, bevacizumab, ranibizumab, and aflibercept. In embodiments, the agent is tocilizumab.

In embodiments, the agent is Nesvacumab (REGN910) (Regeneron monoclonal antibody targeting angiopoietin 2 (Ang2)), REGN2 176-3 (Regeneron monoclonal antibody targeting platelet-derived growth factor receptor-beta (PDGFR-beta) co-formulated with aflibercept), LFG3 16 (Novartis monoclonal antibody targeting complement factor 5 (C5)), R06867461 (Roche bispecific monoclonal antibody targeting VEGF and Ang2), iSONEP (Pfizer/Lpath monoclonal antibody targeting sphingosine-1 -phosphate (SIP)), Teprotumumab (Genmab/River Vision monoclonal antibody targeting insulin-like growth factor- 1 receptor (IGF-1R)), PF582 (Pfenex biosimilar of ranibizumab), ESBA1008 (Alcon single chain fragment antibody targeting VEGF), Abicipar pegol (Allergan DARPin (designed ankyrin repeat proteins) targeting VEGF), or Luminate® (Allegro).

In embodiments, the agent is formulated for intraocular, e.g., intravitreal, administration.

In embodiments, the agent is lampalizumab (anti-Factor D). Lampalizumab is an antigen-binding fragment (Fab) of a humanized monoclonal antibody directed against complement Factor D, which is a rate-limiting enzyme involved in activation of the alternative complement pathway. Genetic polymorphisms and hyperactivity of the alternative complement pathway have been implicated in development of age related macular degeneration and geographic atrophy. Lampalizumab has compound ID: CHEMBL2 109408 in the ChEMBL database.

In some embodiments, the agent comprises a functional fragment of an agent described herein, e.g., a target-binding fragment. For agents that are antibody molecules, the present invention encompasses coupling a target-binding fragment of the antibody molecule to an OHLE polypeptide described herein to produce an improved therapeutic agent with increased ocular retention. In other embodiments, the target-binding fragment comprises the CDRs of the heavy and/or light chain variable domain of the agent. For example, the target-binding fragment comprises the heavy and/or light chain variable domain of the agent, or a sequence that is at least 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto. In some embodiments, the agent can be modified to contain sequence from an IgG2 constant region described herein. Examples and sequences of such agents are provided below.

In embodiments, the agent comprises a heavy and/or light chain variable domain sequence from lampalizumab (e.g., amino acids 1 to 115 of SEQ ID NO:52 and/or amino acids 1 to 110 of SEQ ID NO:53). In embodiments, the agent comprises a heavy and/or light chain variable domain sequence that is at least 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to the corresponding sequence of lampalizumab. In embodiments, the agent comprises a heavy and/or light chain variable domain sequence that differs from the corresponding sequence of lampalizumab by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acids.

In embodiments, the agent comprises a heavy and/or light chain sequence from lampalizumab (e.g., SEQ ID NO:52 and/or SEQ ID NO:53). In embodiments, the agent comprises a heavy and/or light chain sequence that is at least 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to the corresponding sequence of lampalizumab. In embodiments, the agent comprises a heavy and/or light chain sequence that differs from the corresponding sequence of lampalizumab by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acids.

In embodiments, the agent comprises a heavy chain comprising the following sequence EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTG ETTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO:52).

In embodiments, the agent comprises a light chain comprising the following sequence DIQVTQSPSSLSASVGDRVTITCITSTDIDDDMNWYQQKPGKVPKLLISGGNTLRPGVPS RFSGSGSGTDFTLTISSLQPEDVATYYCLQSDSLPYTFGQGTKVEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:53).

In embodiments, the agent is engineered to replace the IgGl portion of the heavy chain sequence with an IgG2 sequence. In embodiments, the engineered agent comprises a heavy chain comprising the following sequence:
EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTG ETTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST FRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:59).

In embodiments, the engineered agent comprises a heavy chain comprising the following Fab sequence:

EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWIN TYTGETTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCEREGGVNNWGQGTL VTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERK (SEQ ID NO:63).

In embodiments, the agent is aflibercept (trade name: Eylea® or Zaltrap). Aflibercept is a recombinant fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgGl . Aflibercept can be formulated as

an iso-osmotic solution for intravitreal administration. Aflibercept is a dimeric glycoprotein with a protein molecular weight of 97 kilodaltons (kDa). Aflibercept can contain glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. Aflibercept can be produced in recombinant Chinese hamster ovary (CHO) cells. See Eylea® FDA Product Label issued October 2014. Aflibercept has compound ID: CHEMBL1742982 in the ChEMBL database.

In embodiments, the agent comprises a sequence from aflibercept (e.g., SEQ ID NO:54). In embodiments, the agent comprises a sequence that is at least 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO:54. In embodiments, the agent comprises a heavy and/or sequence that differs from the corresponding sequence of SEQ ID NO:54 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acids.

In embodiments, the agent comprises the following sequence:

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIW
DSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEK
LVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRS
DQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNH YTQKSLSLSPG (SEQ ID NO:54).

In embodiments, the agent is engineered to replace the IgG1 portion of the sequence with an IgG2 sequence. In embodiments, the engineered agent comprises the following sequence:
SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKG
FIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNC
TARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLY
TCAASSGLMTKKNSTFVRVHEKVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEY
KCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO:60).

In embodiments, the agent is bevacizumab (trade name: Avastin®). Bevacizumab is a recombinant humanized monoclonal IgGl antibody that binds to and inhibits the activity of human vascular endothelial growth factor (VEGF) in *in vitro* and *in vivo* assays. Bevacizumab contains human framework regions and the CDRs of a murine antibody that binds to VEGF. It has a molecular weight of approximately 149 kD. It can be produced in a CHO expression system in a nutrient medium containing gentamicin. See Avastin® FDA Product Label issued August 2014. Bevacizumab has compound ID: CHEMBL1201583 in the ChEMBL database.

In embodiments, the agent comprises a heavy and/or light chain variable domain sequence from bevacizumab (e.g., amino acids 1 to 123 of SEQ ID NO:55 and/or amino acids 1 to 110 of SEQ ID NO:56). In embodiments, the agent comprises a heavy and/or light chain variable domain sequence that is at least 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to the corresponding heavy and/or light chain variable domain sequence of bevacizumab. In embodiments, the agent comprises a heavy and/or light chain variable domain sequence that differs from the corresponding heavy and/or light chain variable domain sequence of bevacizumab by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acids.

In embodiments, the agent comprises a heavy and/or light chain sequence from bevacizumab (e.g., SEQ ID NO:55 and/or SEQ ID NO:56). In embodiments, the agent comprises a heavy and/or light chain sequence that is at least 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to the corresponding heavy and/or light chain sequence of bevacizumab. In embodiments, the agent comprises a heavy and/or light chain sequence that differs from the corresponding heavy and/or light chain sequence of bevacizumab by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acids.

In embodiments, the agent comprises a heavy chain comprising the following sequence:
EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGE
PTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS

FFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO:55).

In embodiments, the agent comprises a light chain comprising the following sequence:
DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO:56).

In embodiments, the agent is engineered to replace the IgGl portion of the heavy chain sequence with an IgG2 sequence. In embodiments, the engineered agent comprises a heavy chain comprising the following sequence:
EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGE
PTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWG
QGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCP
APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKT
KPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:61).

In embodiments, the agent is ranibizumab (trade name: Lucentis®). Ranibizumab is a recombinant humanized IgGl kappa isotype monoclonal antibody fragment. Ranibizumab can be administered intraocularly by intravitreal administration. Ranibizumab lacks an Fc region. Ranibizumab binds to and inhibits human vascular endothelial growth factor A (VEGF-A). Ranibizumab has a molecular weight of approximately 48 kD. Ranibizumab can be produced in an *E. coli* expression system in a nutrient medium containing tetracycline. See Lucentis ® FDA Product Label issued March 2014. Ranibizumab has compound ID: CHEMBL 120 1825 in the ChEMBL database.

In embodiments, the agent comprises a heavy and/or light chain variable domain sequence from ranibizumab (e.g., amino acids 1 to 123 of SEQ ID NO:57 and/or amino acids 1 to 110 of SEQ ID NO:58). In embodiments, the agent comprises a heavy and/or light chain

variable domain sequence that is at least 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to the corresponding heavy and/or light chain variable domain sequence of ranibizumab. In embodiments, the agent comprises a heavy and/or light chain variable domain sequence that differs from the corresponding heavy and/or light chain variable domain sequence of ranibizumab by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acids.

In embodiments, the agent comprises a heavy and/or light chain sequence from ranibizumab (e.g., SEQ ID NO:57 and/or SEQ ID NO:58). In embodiments, the agent comprises a heavy and/or light chain sequence that is at least 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to the corresponding heavy and/or light chain sequence of ranibizumab. In embodiments, the agent comprises a heavy and/or light chain sequence that differs from the corresponding heavy and/or light chain sequence of ranibizumab by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acids.

In embodiments, the agent comprises a heavy chain comprising the following heavy chain Fab sequence:
EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGE
PTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHL
(SEQ ID NO:57).

In embodiments, the agent comprises a light chain comprising the following sequence:
DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:58).

In embodiments, the agent is engineered to replace the IgGl portion of the heavy chain sequence with an IgG2 sequence. In embodiments, the engineered agent comprises a heavy chain comprising the following sequence:
EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGE
PTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWG
QGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCP

APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKT
KPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:62).

In embodiments, the engineered agent comprises a heavy chain comprising the following Fab sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGE
PTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWG
QGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERK (SEQ ID NO:64).

In embodiments, the agent is tocilizumab (trade name: Actemra®). Tocilizumab is a recombinant humanized monoclonal IgGl antibody that binds to and inhibits the activity of Interleukin 6 receptor (IL-6R) in *in vitro* and *in vivo* assays. Tocilizumab contains human framework regions and the CDRs of a murine antibody that binds to IL-6R. It has a predicted molecular weight of approximately 145 kD. See Actemra® FDA Product Label. Tocilizumab has compound ID: CHEMBL1237022 in the ChEMBL database.

In embodiments, the agent comprises a heavy and/or light chain variable domain sequence from tocilizumab (e.g., amino acids 1 to 119 of SEQ ID NO:69 and/or amino acids 1 to 110 of SEQ ID NO:70). In embodiments, the agent comprises a heavy and/or light chain variable domain sequence that is at least 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to the corresponding heavy and/or light chain variable domain of bevacizumab. In embodiments, the agent comprises a heavy and/or light chain variable domain sequence that differs from the corresponding heavy and/or light chain variable domain of bevacizumab by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acids.

In embodiments, the agent comprises a heavy and/or light chain sequence from tocilizumab (e.g., SEQ ID NO:69 and/or SEQ ID NO:70). In embodiments, the agent comprises a heavy and/or light chain sequence that is at least 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to the corresponding heavy and/or light chain sequence of tocilizumab. In embodiments, the agent comprises a heavy and/or light chain sequence that differs from the

corresponding heavy and/or light chain sequence of tocilizumab by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acids.

In embodiments, the agent comprises a heavy chain comprising the following sequence:
QVQLQESGPGLVRPSQTLSLTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYISYSGITTY
NPSLKSRVTMLRDTSKNQFSLRLSSVTAADTAVYYCARSLARTTAMDYWGQGSLVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO:69).

In embodiments, the agent comprises a light chain comprising the following sequence:
DIQMTQSPSSLSASVGDRVTITCRASQDISSYLNWYQQKPGKAPKLLIYYTSRLHSGVPS
RFSGSGSGTDFTFTISSLQPEDIATYYCQQGNTLPYTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO:70).

In embodiments, the agent is engineered to replace the IgGl portion of the heavy chain sequence with an IgG2 sequence. In embodiments, the engineered agent comprises a heavy chain comprising the following sequence:
QVQLQESGPGLVRPSQTLSLTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYISYSGITTY
NPSLKSRVTMLRDTSKNQFSLRLSSVTAADTAVYYCARSLARTTAMDYWGQGSLVTVS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO:71).

Engineering Agents to Increase Systemic Clearance

One problem with certain antibody-based therapeutics that are effective at a specific locus such as the eye, for example in the vitreous, is adverse effects that result from systemic administration. One solution is to provide therapeutics that can be delivered locally as opposed to systemically as exemplified by molecules described herein. Because some therapeutics that are locally delivered, e.g., to the vitreous, will, to some extent, appear systemically, it is advantageous to design a molecule that will have relatively rapid systemic turnover. Applicants have engineered examples of antibodies designed for rapid systemic turnover, e.g., compared to the parental molecule or a reference antibody. This was accomplished by mutating the Fc domain to modify FcRn binding of the molecule, e.g., to reduce FcRn mediated recycling of the antibodies.

In embodiments, the engineered agent comprises an Fc domain comprising a mutation (e.g., 1, 2, 3, or 4 mutations). In embodiments, the mutation is at one or more positions corresponding to H31 1, D313, 1254, or H436 (numbering as in SEQ ID NO:41). In embodiments, the mutation is selected from one or more of H31 1A, H31 IE, H31 IN, D313T, I254A, I254R, and H436A. In embodiments, the engineered agent comprises an Fc domain comprising a mutation corresponding to H31 1A (numbering as in SEQ ID NO:41). In embodiments, the Fc domain is an IgGl Fc domain. In embodiments, the Fc domain is an IgG2 Fc domain.

In embodiments, the Fc domain is a human IgGl Fc domain having the sequence of SEQ ID NO:50 and optionally comprises a mutation at one or more of the underlined positions: (H90, D92, 133, and H215):

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:50).

In embodiments, the IgGl Fc domain comprises a mutation corresponding to one or more of H90A, H90E, H90N, D92T, 133A, I33R, and H215A (numbering according to SEQ ID NO:50).

In embodiments, the Fc domain is a human IgG2 Fc domain having the sequence of SEQ ID NO:5 1 and optionally comprises a mutation at one or more of the underlined positions (H86, D88, I29, and H21 1):

VECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTIS KTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP MLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:51).

In embodiments, the IgG2 Fc domain comprises a mutation corresponding to one or more of H86A, H86E, H86N, D88T, I29A, I29R, and H21 1A (numbering according to SEQ ID NO:51).

In embodiments, the Fc mutation reduces the systemic accumulation (e.g., increases clearance or decreases half life, e.g., the $T_{1/2\beta}$) of the engineered agent. In embodiments, the systemic accumulation is reduced compared with that of another therapeutic agent (e.g., tocilizumab, bevacizumab, ranibizumab, and/or aflibercept). In embodiments, the systemic accumulation is reduced compared with that of tocilizumab and/or aflibercept. In embodiments, the systemic accumulation is reduced compared with the systemic accumulation of a corresponding engineered agent that does not comprise the mutation. In embodiments, the systemic accumulation is assessed following intravitreal administration of the engineered agent.

Eye Diseases

The compositions and methods of the invention are useful in treating ocular diseases, including diseases that primarily affect the eye as well as eye diseases or disorders that are associated with or secondary to other conditions.

In embodiments, the eye disease is macular edema (e.g., diabetic macular edema (DME)), uveitis, dry eye (e.g., dry eye disease or dry eye syndrome), allergic conjunctivitis, ocular pain, macular degeneration (e.g., age related macular degeneration (AMD), e.g., wet AMD or dry AMD), retinopathy (e.g., diabetic retinopathy, e.g., proliferative diabetic retinopathy (PDR)), Rhegmatogenous retinal detachment (RRD), retinal vein occlusion (RVO), neuromyelitis optica (NMO), a back of the eye disease, glaucoma, corneal transplant, corneal abrasion, or physical injury to the eye.

In embodiments, the disease is a back of the eye disease. A back of the eye diseases is an ocular disease of the posterior segment that affects the vasculature and integrity of the retina, macula and/or choroid leading to visual acuity disturbances, loss of sight and/or blindness. Disease states of the posterior segment may result from age, trauma, surgical interventions, and/or hereditary factors. Exemplary back-of-the-eye disease include, but are not limited to, age-related macular degeneration (AMD) cystoid macular edema (CME), diabetic macular edema (DME), posterior uveitis, and/or diabetic retinopathy.

In embodiments, the disease is retinopathy. Retinopathy is damage to the blood vessels of the retina that leads to partial or complete blindness. Symptoms include blurred or dim vision, sudden loss of vision in one or both eyes, seeing black spots, seeing flashing lights, difficulty reading or seeing detailed work, blind spots, seeing distorted shapes, and/or reduced visual sharpness. Retinopathy can be caused by various ophthalmic conditions as well as numerous systemic diseases outside the eye, e.g., diabetes, sickle cell disease, or anemia. Diabetic retinopathy is damage to the blood vessels of the retina as a result of sustained high blood sugar levels arising from diabetes mellitus. Diabetic retinopathy affects more than 80% of all patients who have had diabetes for 10 years or more and is the leading cause of vision loss in developed countries

In embodiments, the disease is glaucoma. Glaucoma affects approximately five percent of persons who are older than 65 years and fourteen percent of those older than 80 years. Glaucoma is the second leading cause of blindness in developed countries. Glaucoma is an optic nerve disorder characterized by cupping of the optic nerve head and loss of peripheral vision. Occasionally there is also loss of central vision. Glaucoma is thought to result from elevated intraocular pressure which results in progressive damage of the optic nerve and consequent loss of retinal ganglion cells.

In embodiments, the disease is macular edema, e.g., diabetic macular edema, non-diabetic macular edema, or macular edema following retinal vein occlusion. Macular edema is an eye disease with symptoms including, but not limited to, distorted vision, blurred vision, loss of vision, protein deposits on or under the macula, accumulation of fluid under the macula, and/or thickening or swelling of the macula. Macular edema involves a buildup of fluid and protein deposits in the macula of the eye, resulting in thickening and swelling of the macula. This thickening and swelling of the macula alters the functional cell relationship in the retina and

promotes an inflammatory reparative response. Macular edema may accelerate the progress of retinopathy, which is characterized by damage to and alteration of the blood vessels of the eye.

Macular edema can be diabetic macular edema (DME) or non-diabetic macular edema. DME occurs in approximately 14% of diabetics. Retinal vascular leakage, associated with diabetic retinopathy (DR), often leads to DME, which is the single greatest cause of vision loss in diabetes. Diabetic macular edema (DME) involves occlusion and leakage of retinal blood vessels, causing reduced visual acuity and potentially blindness. Standard treatments for DME include local administration of steroids or anti-VEGF antibodies. A preferred treatment for macular edema is laser photocoagulation. However, photocoagulation does not yield significant visual improvement in many cases.

In embodiments, the disease is uveitis. Uveitis refers to swelling and irritation of the uvea of the eye. Uveitis is responsible for about 10% of legal blindness in the United States. Symptoms of uveitis include blurred vision; dark, floating spots in the vision; eye pain, redness of the eye; and sensitivity to light. Anterior uveitis involves inflammation in the front of the uvea. Posterior uveitis affects the back the uvea, and involves primarily the choroid. Posterior uveitis affecting primarily the choroid can be referred to as choroiditis. If the retina is also involved, the disease can be referred to as chorioretinitis. Pars planitis is a form of uveitis affecting the pars plana which is between the colored part of the eye (iris) and the choroid. Uveitis can be associated with a variety of disorders including autoimmune disorders or can be idiopathic. Uveitis can be associated with, for example, AIDS, ankylosing spondylitis, Behcet's syndrome, CMV retinitis, herpes zoster infection, histoplasmosis, injury, Kawasaki disease, psoriasis, reactive arthritis, rheumatoid arthritis, sarcoidosis, syphilis, toxoplasmosis, tuberculosis, and ulcerative colitis. Complications include cataracts, glaucoma, fluids within the retina, retinal detachment and vision loss, band keratopathy, retinal edema and/or permanent vision loss. Standard treatments of non-infectious uveitis including corticosteroids and immunosuppressants are limited by significant toxicities, most notably increased intraocular pressure.

In embodiments, the disease is anterior uveitis. In embodiments the disease is posterior uveitis. In embodiments, the disease is choroiditis. In embodiments, the disease is choroiditis. In embodiments, the disease is chorioretinitis. In embodiments, the disease is pars planitis. In

embodiments, the disease is idiopathic uveitis or uveitis associated with an autoimmune disorder. In embodiments, the disease is uveitis associated with AIDS, ankylosing spondylitis, Behcet's syndrome, CMV retinitis, herpes zoster infection, histoplasmosis, injury, Kawasaki disease, psoriasis, reactive arthritis, rheumatoid arthritis, sarcoidosis, syphilis, toxoplasmosis, tuberculosis, or ulcerative colitis.

In embodiments, the disease is macular degeneration, e.g., age related macular degeneration (AMD). Macular degeneration (MD) is a disesase that usually occurs in the elderly (also referred to as age-related macular degeneration (AMD)). The disease involves breakdown of the macula and may result in a loss of vision in the macular due to retinal damage. Symptoms of macular degeneration include straight lines in the field of vision appearing wavy; type in books, magazines and newspapers appearing blurry; and dark or empty spaces block the centre of vision. AMD is the leading cause of vision loss in adults over 50 years old.

AMD can be divided into a dry form affecting 90% of the AMD population and a more rapidly progressing and severe wet form involving blood vessel growth and leakage. Dry AMD has three stages: early, intermediate, and advanced. People with early AMD may not have any symptoms or vision loss. As the disease progresses to the intermediate and advanced forms, patients typically experience a blurred spot in the center of their vision, often called geographic atrophy, due to breakdown of the macula. Wet AMD involves abnormal blood vessel growth and leakage under the macula. The blood and fluid from leaky vessels cause the macula to swell, leading to retinal scarring and rapid vision loss. Although only 10 percent of those with AMD have this type of the disease, wet AMD accounts for 90 percent of all blindness resulting from AMD. There are no approved therapies for dry AMD, although some clinical success has been reported using complement blockers. Wet AMD is primarily treated with intravitreal injections of VEGF-A blockers, such as Lucentis® and Eylea®. However, not all wet AMD patients respond to VEGF-A therapy and some become refractory over time.

In embodiments, the disease is wet AMD or dry AMD. In embodiments, the disease is neovascular (wet) age-related macular degeneration. In embodiments, the disease is dry AMD. In embodiments, the disease is or comprises geographic atrophy.

Models of Eye Disease

The efficacy of compositions and methods described herein can be shown using experimental models of eye disease, e.g., an animal model described herein or known in the art. An experimental autoimmune uveitis model can be employed in rats or mice (Caspi, Invest Ophthalmol Vis Sci 52:1873; Agarwal et al, 900:443-69, 2012) using interphotoreceptor retinoid-binding protein (IRBP) in complete Freund's adjuvant (CFA) immunization. Other models include those known in the art for dendritic cell-induced uveitis, adoptive transfer of cultured effector T cells, spontaneous EAU in IRBP TCR Tg mice, endotoxin-induced uveitis, autoimmune uveoretinitis (Haruta et al., Invest Ophthalmol Vis Sci 53:3264 (201 1); Yoshimura et al, Rheumatology 48:347-354 (2009)).

Other model systems that can be used include, for example, a choroidal neovascularization (CNV) model (Izumi-Nagai et al., Am J Pathol 170:6 (2007); Krzystolik et al., Arch Ophthalmol 120:338 (2002)) and diabetic models such as those described in Kern et al. (Animal Models Of Diabetic Complications Consortium (P01 DK57733), Update Report (September 2001 - January 2004)).

CNV models are representative, e.g., of the human conditions of AMD and DME. Retinal neovascularization models are useful, e.g., for studying ischemic retinopathies, e.g., diabetic retinopathy or retinopathy of prematurity. Various choroidal and retinal neovascularization models are known in the art (see, e.g., Grossniklaus, H.E. et al. Prog Retin Eye Res. 2010 Nov;29(6):500-19. doi: 10.1016/j.preteyeres.2010.05.003. Epub 2010 May 19; Saisin, Y et al. (2003) Journal of Cellular Physiology, 195:241-248; Takahashi, K. et al. (2003) Investigative Ophthalmology & Visual Science, 44(1):409-415; Lima e Silva, R. et al. (2007) FASEB Journal, 21:3219-3230; Tobe et al. (1998) American Journal of Pathology, 153(5): 1641-1646; Dong, A et al. (201 1) PNAS, 108(35): 14614-14619; Dong et al. (2009) J Cell Physiol 219:544-552; Smith, LE et al. 1994 Invest Ophthalmol Vis Sci 1994; 35:101-1 11; Shen, J. et al. (2007) Investigative Ophthalmology & Visual Science, 48(9):4335-4341). Choroidal neovascularization (CNV) can be induced, e.g., by lasers, light, surgery, or genetic modifications. Models of oxygen-induced retinal neovascularization are known in the art and are described, e.g., in Smith, LE et al. 1994 Invest Ophthalmol Vis Sci 1994; 35:101-1 11; Shen, J. et al. (2007) Investigative Ophthalmology & Visual Science, 48(9):4335-4341.

An ischemia/reperfusion model can also be used. See, e.g., Zheng, L et al. Investigative Ophthalmology & Visual Science, vol. 48 no. 1 pp. 361-367, 2007. For example, on Day 1, a 30 gauge needle attached to a fluid bag is inserted into the cornea of anesthetized mice and the intraocular pressure (IOP) is elevated to approximately 120 mmHg to generate ischemia. After 30-90 minutes, the needle is removed, IOP is normalized, and reflow of the retinal circulation occurs. Expression of inflammatory markers including TNF-a and ICAM-1 can be assessed by western blot and qPCR on Day 2-6. Additionally, ganglion cell loss can be assessed by histology on Day 3-14 and capillary degeneration is measured by trypsin digest technique on Day 10-14. For therapeutic studies, test article (e.g., 1 μL with an appropriate concentration of a therapeutic agent) is injected intravitreally either shortly before or after the induction of ischemia.

Generation of antibodies

Antibodies and fragments thereof can be produced using methods known in the art such as monoclonal antibody methodology (e.g., see Kohler and Milstein (1975) Nature 256: 495). Other techniques for producing monoclonal antibodies can also be employed such as viral or oncogenic transformation of B lymphocytes.

Chimeric or humanized antibodies can be prepared based on the sequence of a murine monoclonal antibody prepared using methods known in the art. DNA encoding the heavy and light chain immunoglobulins can be obtained from a murine hybridoma of interest and engineered to contain non-murine (e.g., human) immunoglobulin sequences using standard molecular biology techniques. For example, to create a chimeric antibody, the murine variable regions can be linked to human constant regions using methods known in the art (see e.g., U.S. Pat. No. 4,816,567). To create a humanized antibody, the murine CDR regions can be inserted into a human framework using methods known in the art (see e.g., U.S. Pat. No. 5,225,539, and U.S. Pat. Nos. 5,530,101; 5,585,089; 5,693,762; and 6,180,370).

In embodiments, a human monoclonal antibody is generated using transgenic or transchromosomic mice comprising portions of a human immune system rather than the mouse system. These transgenic and transchromosomic mice include "human Ig mice" such as the HuMAb Mouse® and KM Mouse® (See, e.g., U.S. Pat. Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; U.S. Pat. No.

5,545,807; PCT Publication Nos.: WO 92/03918, WO 93/12227, WO 94/25585, WO 97/13852, WO 98/24884 and WO 99/45962; and PCT Publication No. WO 01/14424).

In embodiments, a human antibody is generated using a mouse that carries human immunoglobulin sequences on transgenes and transchomosomes, such as a mouse that carries a human heavy chain transgene and a human light chain transchromosome. Such mice are described in detail in PCT Publication No. WO 02/43478.

Other transgenic animal systems expressing human immunoglobulin genes are available in the art and can be used. For example, an alternative transgenic system referred to as the Xenomouse™ (Abgenix, Inc.) can be used; such mice are described in, for example, U.S. Pat. Nos. 5,939,598; 6,075,181; 6,1 14,598; 6,150,584; and 6,162,963. Moreover, transchromosomic animal systems expressing human immunoglobulin genes are available in the art. For example, mice carrying both a human heavy chain transchromosome and a human light chain transchromosome are described in Tomizuka et al. (2000, Proc Natl Acad Sci USA 97:722-727). Human monoclonal antibodies can also be prepared using SCID mice into which human immune cells have been reconstituted such that a human antibody response can be generated upon immunization. Such mice are described in, for example, U.S. Pat. Nos. 5,476,996 and 5,698,767.

Phage Display Libraries

In some cases, an antibody or fragment thereof is produced in a method that involves synthesizing a library of human antibodies using phage, screening the library with a target of interest, e.g., a human IL-6, or a fragment thereof, isolating phage that bind the target, and obtaining the antibody from the phage.

Recombinant human antibodies can also be isolated by screening a recombinant combinatorial antibody library. In general, the library is a scFv phage display library, generated using human VL and VH cDNAs prepared from mRNA isolated from B cells. Methods for preparing and screening such libraries are known in the art. Kits for generating phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, catalog no. 27-9400-01; and the Stratagene SurfZAP™ phage display kit, catalog no. 240612). Other methods and reagents that can be used in generating and screening antibody display libraries are known in the art (see, e.g., U.S. Pat. No. 5,223,409; PCT Publication Nos. WO 92/18619, WO 91/17271, WO 92/20791, WO 92/15679, WO 93/01288, WO 92/01047, WO

92/09690; Fuchs et al, Bio/Technology 9:1370-1372 (1991); Hay et al, Hum Antibod Hybridomas 3:81-85 (1992); Huse et al, Science 246:1275-1281 (1989); McCafferty et al, Nature 348:552-554 (1990); Griffiths et al, EMBO J 12:725-734 (1993); Hawkins et al, J Mol Biol 226:889-896 (1992); Clackson et al, Nature 352:624-628 (1991); Gram et al, Proc Natl Acad Sci USA 89:3576-3580 (1992); Garrad et al, Bio/Technology 9:1373-1377 (1991); Hoogenboom et al, Nuc Acid Res 19:4133-4137 (1991); and Barbas et al, Proc Natl Acad Sci USA 88:7978-7982 (1991), all incorporated herein by reference.

In an example for isolating and producing antibodies to a particular target with desired characteristics, a human antibody is first used to select human heavy and light chain sequences having similar binding activity toward the target, using epitope imprinting methods described in PCT Publication No. WO 93/06213, incorporated herein by reference. The antibody libraries used in this method are generally scFv libraries prepared and screened as described in PCT Publication No. WO 92/01047; McCafferty et al, Nature 348:552-554 (1990); and Griffiths et al., EMBO J 12:725-734 (1993), all incorporated herein by reference.

Once initial human VL and VH domains are selected, "mix and match" experiments are performed, in which different pairs of the initially selected VL and VH segments are screened for target binding to select preferred VL/VH pair combinations. To select for desirable features of an antibody, the VL and/or VH segments of a selected pair can be randomly mutated. This *in vitro* affinity maturation can be accomplished, for example, by amplifying VH and VL domains using PCR primers complimentary to a CDR of one or both of the selected VH and VL domains, which primers contain a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode VH and VL segments into which random mutations have been introduced into the VH and/or VL. Such randomly mutated VH and VL segments can be re-screened for binding to the target.

Following screening and isolation of an antibody from a recombinant immunoglobulin display library, nucleic acids encoding the selected antibody can be recovered from the display package (e.g., from the phage genome) and subcloned into other expression vectors using recombinant DNA techniques known in the art. Such antibodies can be further manipulated to produce an antibody, antibody fragment, or other therapeutic agent such as those described herein.

Administration of Compositions

A engineered agent or composition described herein (e.g., a composition comprising an engineered agent described herein) can be administered, e.g., to the eye, via any known administration route. In embodiments, the composition is delivered locally, either in direct contact with or near a cell or tissue being targeted. Non-limiting examples of delivery methods include injection, infusion, or implantation, e.g., delivery via an implanted device. In embodiments, the composition is administered topically. In embodiments, the composition is administered by injection (e.g., intraocular injection, e.g., intravitreal injection).

In embodiments, the engineered agent composition comprising the engineered agent is administered intraocularly, e.g., intravitreally (e.g., via intravitreal injection, an ophthalmic insert or device, or genetic delivery).

In some embodiments, the composition is administered as an ophthalmic formulation which optionally comprises one or more pharmaceutically acceptable carriers or excipients. The methods can comprise administration of the composition and an ophthalmically acceptable carrier. In some embodiments, the ophthalmic formulation is a liquid, semi-solid, insert, film, microparticle, or nanoparticle.

In some embodiments, the composition is formulated for intravitreal administration (e.g., intravitreal injection).

In some embodiments, the composition is formulated for topical administration, e.g., to the eye. The topical formulation can be a liquid formulation or semi-solid, for example, a topical formulation can include an aqueous solution, an aqueous suspension, an ointment or a gel. An ophthalmic formulation can be topically applied to the front of the eye, under the upper eyelid, on the lower eyelid and in the cul-de-sac. Typically, the ophthalmic formulation is sterile. An ophthalmic formulation can contain one or more pharmaceutical excipients suitable for the preparation of ophthalmic formulations. Examples of such excipients are preserving agents, buffering agents, chelating agents, antioxidant agents and salts for regulating the osmotic pressure. Ophthalmic formulations, including both ointments and suspensions, typically have a viscosity that is suited for the selected route of administration.

In some embodiments, the formulation is a liquid formulation comprising a polymer. Such a polymer can be used to improve the bioavailability, raise viscosity, or reduce drainage from the eye of a liquid formulation. Suitable polymers include, but are not limited to, those

described in Wagh et al. (Asian J Pharm, 2:12-17, 2008). In non-limiting examples, the polymer is sodium hyaluronase, chitosan, a cyclodextrin (e.g., hydroxypropyl -P-cyclodextrin), polygalactoronic acid, xyloglucan, xanthan gum, gellan gum, a thiomer, a poly(ortho ester) (e.g., Einmahl, Adv Drug Deliv Rev 53:45-73, 2001), or a tamarind seed polysaccharide (e.g., Ghelardi et al, Antimicrob Agents Chemother 48:3396-3401, 2004).

In some embodiments, a formulation comprising a composition for ophthalmic delivery can comprise one or more of surfactants, adjuvants, buffers, antioxidants, tonicity adjusters, preservatives (e.g., EDTA, BAK (benzalkonium chloride), sodium chlorite, sodium perborate, polyquaterium-1), thickeners or viscosity modifiers (e.g., carboxymethyl cellulose, hydroxymethyl cellulose, polyvinyl alcohol, polyethylene glycol, glycol 400, propylene glycol hydroxymethyl cellulose, hydroxpropyl-guar, hyaluronic acid, and hydroxypropyl cellulose) and the like. Additives in the formulation may include, but are not limited to, sodium chloride, sodium bicarbonate, sorbic acid, methyl paraben, propyl paraben, chlorhexidine, castor oil, and sodium perborate.

In some embodiments, purified or deionized water is used in the composition. The pH can be adjusted by adding any physiologically and ophthalmically acceptable pH adjusting acids, bases or buffers to within the range of about 5.0 to 8.5, e.g., pH 7.0, pH 7.3, pH, 7.4, or pH 7.5. Ophthalmically acceptable examples of acids include acetic, boric, citric, lactic, phosphoric, hydrochloric, and the like, and examples of bases include sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate, tromethamine, trishydroxymethylamino-methane, and the like. Examples of salts and buffers that can be used in a formulation include citrate/dextrose, sodium bicarbonate, ammonium chloride and mixtures of the aforementioned acids and bases.

In some embodiments, the osmotic pressure of the ophthalmic composition may be from about 10 milliosmolar (mOsM) to about 400 mOsM, for example, 200 to 400 mOsM, or 220 to 370 mOsM. Generally, the osmotic pressure can be adjusted using physiologically and ophthalmically acceptable salts or excipients. In some embodiments, sodium chloride is included in a formulation, for example, sodium chloride is present in a formulation in a concentration ranging from 0.01% to 1% by weight, or from 0.05% to 0.45%> by weight, based on the total weight of the composition. Equivalent amounts of one or more salts made up of cations such as potassium, ammonium and the like and anions such as chloride, citrate, ascorbate, borate,

phosphate, bicarbonate, sulfate, thiosulfate, bisulfate, sodium bisulfate, ammonium sulfate, and the like can also be used in addition to or instead of sodium chloride to achieve osmolalities within the desired range. In some embodiments, a sugar such as mannitol, dextrose, sorbitol, glucose and the like is used to adjust osmolality.

In some embodiments, the methods involve forming or supplying a depot of the agent in contact with the external surface of the eye. A depot refers to a source of agent that is not rapidly removed by tears or other eye clearance mechanisms. This allows for continued, sustained high concentrations of agent be present in the fluid on the external surface of the eye by a single application. In some embodiments, the depot can remain for up to eight hours or more. In some embodiments, the ophthalmic depot formulation includes, but is not limited to, aqueous polymeric suspensions, ointments, and solid inserts.

In some embodiments, a semi-solid composition is a liquid formulation that increases in viscosity upon application to the eye, typically due to the presence of a polymer in the liquid formulation for which an increase is viscosity occurs with a change in temperature, pH, or electrolyte concentration. The polymer can be, for example, celluloseacetophthalate, polyacrylic acid, gellan gum, hyaluronase, chitosan, salts of alginic acid (e.g., sodium alginate), or a block copolymer of ethylene oxide and propylene oxide (e.g., Pluronic®, BASF; poloxamer). In some embodiment, the polyacrylic acid is cross-linked acrylic acid (e.g., Carbopol®). In some embodiments, the semi-solid composition comprises a mixture of carbopol and a block copolymer of ethylene oxide and propylene oxide; a mixture of methyl cellulose and hydroxyethyl cellulose; or a mixture of polyethylene glycol and a block copolymer of ethylene oxide and propylene oxide.

In some embodiments, the ophthalmic formulation is an ointment or gel. In some embodiments, the ophthalmic formulation is an oil-based delivery vehicle. For example, the formulation can comprise a petroleum or lanolin base to which the agent is added (for example at 0.1 to 2%), optionally together with one or more further excipients. Common bases can include, but are not limited to, mineral oil, petrolatum and combinations thereof. In some embodiments, the ointment is applied as a ribbon onto the lower eyelid.

In some cases, the ophthalmic composition is an ophthalmic insert. In embodiments, the composition is administered intravitreally via an ophthalmic insert.

For example, the ophthalmic insert is biologically inert, soft, bio-erodible, viscoelastic, stable to sterilization after exposure to therapeutic agents, resistant to infections from air borne bacteria, bio-erodible, biocompatible, and/or viscoelastic. In some embodiments, the insert comprises an ophthalmically acceptable matrix, e.g., a polymer matrix. The matrix is typically a polymer and the IL-6a composition is dispersed within the matrix or bonded to the polymer matrix. In some embodiments, the agent is slowly released from the matrix through dissolution or hydrolysis of a covalent bond. In some embodiments, the polymer is bioerodible (soluble) and the dissolution rate thereof can control the release rate of the agent dispersed therein. In another form, the polymer matrix is a biodegradable polymer that breaks down such as by hydrolysis to thereby release the agent bonded thereto or dispersed therein. In further embodiments, the matrix and agent can be surrounded with an additional polymeric coating to further control release. In some embodiments, the insert comprises a biodegradable polymer such as polycaprolactone (PCL), an ethylene/vinyl acetate copolymer (EVA), polyalkyl cyanoacrylate, polyurethane, a nylon, or poly(dl-lactide-co-glycolide) (PLGA), or a copolymer of any of these. In some cases, the agent is dispersed into the matrix material or dispersed amongst the monomer composition used to make the matrix material prior to polymerization. In some embodiments, the amount of agent is from about 0.1 to about 50%, or from about 2 to about 20%. The biodegradable or bioerodible polymer matrix can be used so that the spent insert does not have to be removed from the eye. As the biodegradable or bioerodible polymer is degraded or dissolved, the agent is released.

In further embodiments, the ophthalmic insert comprises a polymer, including, but are not limited to, those described in Wagh, et al, "Polymers used in ocular dosage form and drug delivery systems", Asian J. Pharm., pages 12-17 (January 2008), which is incorporated herein by reference in its entirety. In some embodiments, the insert comprises a polymer selected from polyvinylpyrrolidone (PVP), an acrylate or methacrylate polymer or copolymer (e.g., Eudragit® family of polymers from Rohm or Degussa), hydroxymethyl cellulose, polyacrylic acid, poly(amidoamine) dendrimers, poly(dimethylsiloxane), polyethylene oxide, poly(lactide-co-glycolide), poly(2-hydroxyethylmethacrylate), polyvinyl alcohol), or poly(propylene fumarate). In some embodiments, the insert comprises Gelfoam®. In some embodiments, the insert is a polyacrylic acid of 450 kDa-cysteine conjugate.

The insert can comprise a core that contains the composition and an outer tube (e.g., as described in U.S. Patent Pub. No. 20040009222). In some cases, the outer tube can be permeable, semi-permeable, or impermeable to the drug. In some embodiments, the core includes a polymer matrix that does not have a significant effect on the rate of release. In some cases, the outer tube, the polymer matrix of the core, or both is bioerodible. The co-extruded product can be segmented into drug delivery devices. In some embodiments, the device is uncoated so that the respective ends are open, or the device is coated with, for example, a layer that is permeable to the composition, semi-permeable to the composition, or bioerodible. In certain embodiments, the composition and at least one polymer are admixed in powder form.

In some embodiments, the ophthalmic composition is an ophthalmic film. Polymers suitable for such films include, but are not limited to, those described in Wagh, et al. *{supra).* In some embodiments, the film is a soft-contract lens, for example, a lens composed of copolymers of N,N-diethylacrylamide and methacrylic acid cross-linked with ethyleneglycol dimethacrylate.

In certain embodiments, the composition is in an insert that is in a tubular form, and may be segmented.

In some embodiments, the composition is formulated in a therapeutically effective amount, coated by or dispersed in a polymer matrix, such that the composition is in granular or particulate form. In some embodiments, the composition is released from the formulation as drug from the granules dissolves into or within the matrix, diffuses through the matrix, and is released into the surrounding physiological fluid. In some embodiments, the rate of release is limited primarily by the rate of dissolution of the composition from the granules/particles into the matrix; the steps of diffusion through the matrix and dispersion into the surrounding fluid are primarily not release-rate-limiting. In certain embodiments, the polymer matrix is non-bioerodible, while in other embodiments it is bioerodible. Exemplary non-bioerodible polymer matrices can be formed from polyurethane, polysilicone, poly(ethylene-co-vinyl acetate) (EVA), polyvinyl alcohol, and derivatives and copolymers thereof. Exemplary bioerodible polymer matrices can be formed from polyanhydride, polylactic acid, polyglycolic acid, polyorthoester, polyalkylcyanoacrylate, and derivatives and copolymers thereof.

In some cases, the composition is formulated in a collagenous material. For example, the insert can be a soluble ophthalmic drug insert (e.g., a polymeric oval film that can be introduced in the upper conjuctival sac for drug delivery; an elliptical insert such as OCUSERT®

(pilocarpine ocular therapeutic system, developed by Alza Corporation) which is made of ethylene vinyl acetate; Lacrisert®, a rod shaped insert made of cellulose; New Ophthalmic Drug Delivery Systems (NODS), made of poly(vinyl alcohol); or inserts such as those described in Fabrizio (Adv Drug Deliv Rev 16: 95-106, 1998). In some cases, the insert comprises collagen, gelatin, or a polymer, wherein the polymer is selected from polycaprolactone (PCL), an ethylene/vinyl acetate copolymer (EVA), polyalkyl cyanoacrylate, polyurethane, a nylon, poly(dl-lactide-co-glycolide) (PLGA), or a copolymer of any of these. In some cases, the insert is implanted under the upper eyelid. In some cases, the insert is implanted in the posterior segment of the eye, in the choroidal space, or in the sclera. In some embodiments, the insert is implanted intravitreally or sub-retinally. In some embodiments, the insert is injected sub-retinally. Methods of administration and techniques for their preparation are set forth in Remington's: The Practice of Science of Pharmacy, 20th edition (Lippincott Williams & Wilkins, 2006), which is incorporated herein by reference in its entirety.

In other embodiments, an insert containing a composition described herein provides a sustained release of the agent to the vitreous of the eye. As used herein, "sustained release" means that the composition releases the agent over an extended period of time in a controlled fashion. In some embodiments, the insert releases the agent at a rate such that the aqueous agent concentration remains less than the vitreous agent concentration during the release. In some embodiments, the aqueous agent concentration is from about 0.002 μg/mL to about 0.01 μg/mL or from about 0.01 μg/mL, to about 0.05 μg/mL, or less than about 0.05 μg/mL. In some embodiments, the agent is released at a rate of about 1 μg/day to about 50 μg/day, or from about 1 μg/day to about 10 μg/day. In some embodiments, the insert further comprises an additional therapeutic agent, e.g., fluocinolone acetonide (such as that found in the ophthalmic insert Retisert®).

In some embodiments, the ophthalmic composition comprises microspheres or nanoparticles. In some embodiment, the microspheres comprise gelatin. In some embodiments, the microspheres are injected to the posterior segment of the eye, in the choroidal space, in the sclera, intravitreally or sub-retinally. In some embodiments, the microspheres or nanoparticles comprise a polymer including, but not limited to, those described in Wagh, et al. (Asian J Pharm 2:12-17, 2008). In some embodiments, the polymer is chitosan, a polycarboxylic acid such as polyacrylic acid, albumin particles, hyaluronic acid esters, polyitaconic acid,

poly(butyl)cyanoacrylate, polycaprolactone, poly(isobutyl)caprolactone, poly(lactic acid-co-glycolic acid), or poly(lactic acid). In some embodiments, the microspheres or nanoparticles comprise solid lipid particles.

In some embodiments, the composition comprises an ion-exchange resin. In some embodiments, the ion-exchange resin is an inorganic zeolite or synthetic organic resin. In some embodiments, the ion-exchange resin includes, but is not limited to, those described in Wagh, et al., *supra*, which is incorporated herein by reference in its entirety. In some embodiments, the ion-exchange resin is a partially neutralized polyacrylic acid.

The composition can be provided in an aqueous polymeric suspension. In some embodiments, the composition or a polymeric suspending agent is suspended in an aqueous medium (e.g., having the properties as described above). Examples of polymeric suspending agents include, but are not limited to, dextrans, polyethylene glycols, polyvinylpyrolidone, polysaccharide gels, Gelrite®, cellulosic polymers like hydroxypropyl methylcellulose, and carboxy-containing polymers such as polymers or copolymers of acrylic acid, as well as other polymeric demulcents. In some embodiments, the polymeric suspending agent is a water swellable, water insoluble polymer, especially a cross-linked carboxy-containing polymer. In some embodiments, the polymeric suspending agent comprises from at least about 90% to about 99.9%, or from about 95% to about 99.9%, by weight based on the total weight of monomers present, of one or more carboxy-containing monoethylenically unsaturated monomers. In some embodiments, the carboxy-containing monoethylenically unsaturated monomer includes acrylic acid, methacrylic acid, ethacrylic acid, methylacrylic acid (crotonic acid), cis-.alpha.-methylcrotonic acid (angelic acid), trans-a-methylcrotonic acid (tiglic acid), a-butylcrotonic acid, .alpha.-phenylacrylic acid, a-benzylacrylic acid, a-cyclohexylacrylic acid, phenylacrylic acid (cinnamic acid), coumaric acid (o-hydroxycinnamic acid), and umbellic acid (p-hydroxycoumaric acid). In some embodiments, the polymer is cross-linked by a polyfunctional crosslinking agent (e.g., a difunctional crosslinking agent). In some embodiments, the crosslinking agent is contained in an amount of from about 0.01% to about 5%, or from about 0.1% to about 5.0%, or from about 0.2% to about 1%, based on the total weight of monomers present. In some embodiments, the crosslinking agents are nonpolyalkenyl polyether difunctional crosslinking monomers such as divinyl glycol, 2,3-dihydroxyhexa-l,5-diene, 2,5-dimethyl-l,5-hexadiene, divinylbenzene, N,N-diallylacrylamide, N,N-diallymethacrylamide; polyalkenyl

polyether crosslinking agents containing two or more alkenyl ether groupings per molecule, e.g., alkenyl ether groupings containing terminal $H_2C=C$ groups, prepared by etherifying a polyhydric alcohol containing at least four carbon atoms and at least three hydroxyl groups with an alkenyl halide such as allyl bromide or the like, e.g., polyallyl sucrose, polyallyl pentaerythritol, or the like; diolefmic non-hydrophilic macromeric crosslinking agents having molecular weights of from about 400 to about 8,000, such as insoluble diacrylates and polyacrylates and methacrylates of diols and polyols, diisocyanate hydroxyalkyl acrylate or methacrylate reaction products of isocyanate terminated prepolymers derived from polyester diols, polyether diols or polysiloxane diols with hydroxyalkylmethacrylates, and the like.

In some embodiments, the cross-linked polymers are made from a carboxy-containing monoethylenically unsaturated monomer or monomers as the sole monoethylenically unsaturated monomer present, together with a crosslinking agent or agents. In some embodiments, the polymers are ones in which up to about 40%, and preferably from about 0% to about 20% by weight, of the carboxy-containing monoethylenically unsaturated monomer or monomers has been replaced by one or more non-carboxyl-containing monoethylenically unsaturated monomer or monomers containing only physiologically and ophthalmically innocuous substituents, including acrylic and methacrylic acid esters such as methyl methacrylate, ethyl acrylate, butyl acrylate, 2-ethylhexylacrylate, octyl methacrylate, 2-hydroxyethylmethacrylate, 3-hydroxypropylacrylate, and the like, vinyl acetate, N-vinylpyrrolidone, and the like (e.g., Mueller et al. U.S. Pat. No. 4,548,990). In some embodiments, the polymers include polycarbophil (Noveon AA-1), Carbopol®, and DuraSite®. In some embodiments, the cross-linked polymers are prepared by suspension or emulsion polymerizing the monomers, using conventional free radical polymerization catalysts, to a dry particle size of not more than about 50 μιη in equivalent spherical diameter. In some embodiments, the average dry particle size is from about 1 to about 30 μιη, or from about 3 to about 20 μιη in equivalent spherical diameter. In some embodiments, the polymer particles are obtained by mechanically milling larger polymer particles. In further embodiments, such polymers will have a molecular weight from about 250,000 to about 4,000,000, and from 3,000,000,000 to 4,000,000,000. In other embodiments, the particles of cross-linked polymer are monodisperse, meaning that they have a particle size distribution such that at least about 80%, about 90% or about 95%, of the particles fall within a μιη band of major particle size distribution. In further embodiments, the monodisperse particle size means that there

is no more than about 20%, about 10%, or about 5% particles of a size below Iμιη. In some embodiments, the aqueous polymeric suspension comprises from about 0.05 to about 1%, from about 0.1 to about 0.5%, or from about 0.1 to about 0.5%, of the agent and from about 0.1 to about 10%, from about 0.5 to about 6.5%, from about 0.5 to about *2.0%,* from about 0.5%> to about 1.2%, from about 0.6 to about 0.9%, or from about 0.6 to about 0.8% of a polymeric suspending agent. Although referred to in the singular, it should be understood that one or more species of polymeric suspending agent can be used with the total amount falling within the stated ranges. In one embodiment, the amount of insoluble lightly cross-linked polymer particles, the pH, and the osmotic pressure can be correlated with each other and with the degree of crosslinking to give a composition having a viscosity in the range of from about 500 to about 100,000 centipoise, and preferably from about 1,000 to about 30,000 or about 1,000 to about 10,000 centipoise, as measured at room temperature (about 25°C.) using a Brookfield Digital LVT Viscometer equipped with a number 25 spindle and a 13R small sample adapter at 12 rpm. In some embodiments, the viscosity is from about 10 to about 400 centipoise, from about 10 to about 200 centipoises or from about 10 to about 25 centipoise.

In some embodiments, the aqueous polymeric suspensions may be formulated so that they retain the same or substantially the same viscosity in the eye that they had prior to administration to the eye. In some embodiments, they may be formulated so that there is increased gelation upon contact with tear fluid. For instance, when a formulation containing DuraSite® or other similar polyacrylic acid-type polymer is administered to the eye at a pH of less than about 6.7, the polymer may swell upon contact with tear fluid since it has a higher pH (around 7). This gelation or increase in gelation may lead to entrapment of the suspended particles, thereby extending the residence time of the composition in the eye. In some embodiments, the agent is released slowly as the suspended particles dissolve over time. In some embodiments, this delivery route increases patient comfort and increased agent contact time with the eye tissues, thereby increasing the extent of drug absorption and duration of action of the formulation in the eye. The agents contained in these drug delivery systems will be released from the gels at rates that depend on such factors as the drug itself and its physical form, the extent of drug loading and the pH of the system, as well as on any drug delivery adjuvants, such as ion exchange resins compatible with the ocular surface, which may also be present.

In some embodiments, an engineered agent is provided to a subject using genetic delivery, e.g., local genetic delivery. In embodiments, the genetic delivery is accomplished using a gene therapy vector, e.g., an adeno-associated virus (AAV) or lentivirus. Such delivery can be via a transient expression system, a stable (e.g., integrated) expression system such as a lentiviral delivery system manufactured by Bluebird Bio (Cambridge, MA), or delivery in a cell factory such as those manufactured by Neurotech (Cumberland, Rhode Island).

All technical features can be individually combined in all possible combinations of such features.

EQUIVALENTS

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein.

EXAMPLES

The following non-limiting examples further illustrate embodiments of the inventions described herein.

Example 1: Validation of local IL-6 blockade in choroidal neovascularization (CNV) model

To determine whether local IL-6 blockade could be effective for treating eye disease, e.g., diabetic macular edema (DME) or wet AMD, an anti-IL-6 antibody was locally administered using a model system for choroidal neovascularization. A laser-induced CNV model (eyecro.com/in-vivo/laser-induced-choroidal-neovascularization-cnv/) reproduces many of the pathologic processes underlying DME including inflammation and angiogenesis. Studies were performed in rats at EyeCRO (Oklahoma City, OK). Six animals in each group underwent bilateral laser treatment on Day 0 to produce three lesions per eye. On days 3 and 10, 3 μg of a polyclonal anti-rat-IL-6 antibody (R&D Systems AF506; Minneapolis, MN) was administered to the test group by intravitreal (IVT) injection, while PBS or an anti-VEGF polyclonal antibody (R&D Systems AF564) was administered to the vehicle and positive control groups, respectively. *In vivo* angiography was performed on days 15 and 22 to measure the lesion area. On both days 15 and 22, the anti-IL-6 treated group had significantly reduced neovascularization

compared to the vehicle control. There was no significant difference in response between the anti-IL-6 treated group and the anti-VEGF positive control. Fig. 1 shows the results of such an experiment. These data demonstrate that an IL-6a, e.g., an anti-IL6 antibody, administered IVT can reduce neovascularization in a rat CNV model to similar levels as an anti-VEGF positive control ($p = 0.0054$ on Day 15 and $p = 0.0005$ on Day 22 for anti-IL-6 vs. vehicle control).

These data indicate that local blockade of IL-6 can be useful for treating eye disease such as diseases involving vascular leakage, e.g., macular edema.

Example 2: Candidate antibody IL-6 antagonists

Candidate antibody IL-6 antagonists were developed using a process that first involved immunizations. Immunizations were performed at the direction of the inventors by a contract research organization (CRO). Five BALB/C mice were injected subcutaneously with 80 µg human IL-6 (R&D Systems, cat# 206-IL/CF, Minneapolis, MN) in PBS containing 1 M NaCl with Freud's adjuvant. Two boosts were performed with 80 µg and 50 µg IL-6. Spleen cells were harvested from the highest titer mouse and fused with P3x763Ag8.653 myeloma cells to form hybridomas.

Hybridoma supernatants were screened for IL-6 binding and antagonism. For the binding ELISA, Costar 9018 plates were coated with 1 µg/mL human IL-6 in PBS overnight at 4°C. Wells were blocked with PBS containing 2% BSA, washed, and then incubated with 50 µï¿½ of each hybridoma supernatant diluted 1:2 with PBS containing 2% BSA. After 60 minutes, wells were washed three times with 300 µï PBS containing 0.1% Tween-20. Anti-mouse-HRP diluted 1:3000 in PBS-BSA was then added to each well and incubated for 30 minutes. Wells were washed as above then 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added and the signal measured at 450 and 550 nm. For antagonism studies, HEK-Blue™-IL6 reporter cells (InvivoGen, San Diego, CA) were incubated with increasing concentrations of human IL-6 in the presence of 1:10 diluted hybridoma supernatant. After 20-24 hours, 20 µï of supernatant was mixed with 180 µï QuantiBlue™ (InvivoGen) and the absorbance measured at 655 nm.

Based on binding and antagonism studies, hybridoma 64 was selected by applicants as a lead and subcloned at the CRO. Hybridoma 64 (a murine monoclonal) was further tested for the ability to inhibit binding of IL-6/IL-6Ra complex to gpl30 using an enzyme-linked

immunosorbant assay (ELISA). Hybridoma 64 at a concentration of 1.5 µg/ηι1significantly reduced binding of an IL-6/IL-6Ra complex to immobilized gpl30 by ELISA (Fig. 2).

The subclones were rescreened and the variable domains of subclone 64.58 were amplified by 5' RACE PCR and sequenced. The mouse variable domain sequences (referred to as m64) are as follows:

m64 VH (variable heavy chain)
QVQLQQSGAELVRPGTSVKVSCKASGYAFSNYLIEWVKQRPGQGLEWIGVITPGSGTIN YNEKFKGKAVLTADKSSSTVYMQLSSLTSDDSAVYFCAKSRWDPLYYYALEYWGQGT SVTVSS (SEQ ID NO: 13)

m64 VL (variable light chain)
DIVLTQSPASLAVSLGQRATISCRASESVDNYGISFMNWFQQKPGQPPKLLIYAASNQGS GVPARFSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPLTFGAGTKLELK (SEQ ID NO: 14)

To create humanized sequences, the m64 complementarity determining regions (CDRs) were grafted into a human germline framework selected for similarity to the mouse sequence by a computational algorithm. The humanized sequences (referred to as h64) were as follows (altered residues compared to the m64 sequences are underlined) and have about 79.5% identity (VH) and 84.4% identity (VL) with the murine sequences:

h64 VH
QVQLVQSGAEVKKPGSSVKVSCKASGYAFSNYLIEWVRQAPGQGLEWMGVITPGSGTI NYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSRWDPLYYYALEYWGQGT !VTVSS (SEQ ID NO: 15)

h64 VL
DIVMTQSPDSLAVSLGEPvATINCRASESVDNYGISFMNWYQQKPGQPPKLLIYAASNQG SGVPDRFSGSGSGTDFTLTI SSLOAEDVAVYYCQQSKEVPLTFGQGTKLEIK (SEQ ID NO:16)

The humanized sequences were synthesized by DNA2.0 (Menlo Park, CA), then cloned into pcDNA3.1-derived expression vectors as inline fusions with the human IgGl constant domains. IgGs were expressed by transient transfection in Freestyle™-293 cells (Invitrogen, Grand Island, NY) and purified by protein-A chromatography. In both binding and antagonism studies, the h64 IgG demonstrated considerably reduced potency compared to its m64 predecessor. Therefore, yeast display was utilized to restore the lost affinity.

To carry out the affinity maturation designed to restore or improve the affinity of the humanized h64IgG, the h64 antibody sequences were recloned to generate a Fab molecule in pYC2/CT-derived yeast vectors in which the FabH chain was fused to the anti-FITC scFv 4m5.3 through a (G4S)3 linker (SEQ ID NO: 29). A library of h64 variants was then generated by error prone PCR following the protocol of Chao et al. (2006, Nature Protocols, 1:755-768). H64 variants were expressed and surface captured by yeast labeled with FITC-PEG-NHS then incubated with biotinylated human IL-6. Bound IL-6 was detected with streptavidin-APC, and cells with the highest amount of bound IL-6 relative to the amount of displayed Fabs were selected on a BD FACSAria™ cell sorter. After four rounds of selection, a population of higher affinity variants was selected and sequenced. The sequence of the clone selected by affinity maturation (referred to as h64-1.4) is as follows with the selected mutations (i.e., mutated compared to the sequences of h64 VH and VL) in boldface and the CDRs are underlined. These are the variable domains of 018 (as well as the 020 and 029 IL-6a molecules described below). Note that the full Fabs include the CK and IgGl CHI domains. In the context of this application, reference to a "Fab" heavy chain or light chain amino acid sequence means that sequence can be part of a functioning Fab consisting of a light chain-derived sequence and a heavy chain-derived sequence.

h64-1.4 VH (018VH)(variable domain)
QVQLVQSGAEVKKPGSSVKVSCKAS GYALSNYLIE WVRQAPGQGLEWMG VITPGSGTI NYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCA RSRWDPLYYYALEY WGQGT TVTVSS (SEQ ID NO: 17)

h64-1.4 VL (018VL) (variable domain)

DIVMTQSPDSLAVSLGERATINC RASESVDNYGIPFMN WYQQKPGQPPKLLIY AASNRG SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYY CQQSEEVPLT FGQGTKLEIKRTV (SEQ ID NO: 18)

The h64-1.4 variable domains were recloned into the pcDNA3.1 human IgGl vector and expressed as a full length IgGl in Freestyle™-HEK293 cells (Life Technologies). The resulting purified IgG was significantly more potent than the original h64 antibody in both binding and cellular antagonism studies. Testing affinity using the yeast system, the affinity increased from 343 pM for the original humanized molecule to 43 pM. The antagonist potency was about a ten-fold increase as assayed using the HEK-Blue cell system.

The h64-l .4 IgG was reformatted as a Fab for use in ocular and other indications. Additionally, another round of library generation and yeast based selections was performed to further improve affinity. After four rounds of selection, there was significant enrichment for a VH variant with the A79V mutation. Antibodies, variants and fragments thereof comprising the A79V variant are referred to as 019 IL-6a antibodies, variants, and fragments thereof.

Example 3: Format selection

To investigate suitable formats for an antibody-based IL-6 antagonist, IL-6 antibodies selected as described *supra* were tested for transient expression, stability, aggregation properties, binding affinity, and IC50 using Fab, $SCFV(V_H\text{-}V_L)$ and $SCFV(V_L\text{-}V_H)$ forms of the 018 sequences.

Results of these studies for one of the candidate IL-6a molecules (sequences containing the 018 variable region) are shown in Table 1.

Table 1

Parameter	**Fab**	**scFv(V_H-V_L)**	**scFv(V_L-V_H)**
Transient expression	45 mg/ml	2 mg/L	4mg/L
Stability (T_M)	73°C	43°C	46°C
Aggregation (SEC, MALS)	No	Yes	N/A
Binding affinity (K_D)	240 pM	1 nM	720 pM
IC50 with 10 pM IL-6	255 pM	160 pM	125 pM

These data demonstrate a method of identifying key features of various formats of an antibody-based IL-6 antagonist and illustrates that for IL-6 antagonists containing the 018 variable regions, the 018Fab format has the most favorable features in most key categories, i.e., expression, stability, aggregation, and binding affinity compared to an scFv configuration. The IC50 of the 018 Fab falls within a reasonable range for therapeutic use.

Example 4: Examples of IL-6a antibodies, fragments, and derivatives

Applicants have identified the following sequences using methods described herein. Underlined sequences represent CDRs of the heavy and light chains. Other sequences can be found throughout the specification.

018 Heavy chain (full length; fl018HC) polypeptide sequence in an IgGl framework

QVQLVQSGAEVKKPGSSVKVSCKAS GYALSNYLIE WVRQAPGQGLEWMG VITPGSGTI NYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCA RSRWDPLYYYALEY WGQGT TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 19)

018 Heavy chain (full length; fl018HC) nucleic acid sequence in an IgGl framework

CAAGTGCAGCTGGTGCAGTCAGGGGCCGAGGTTAAGAAGCCAGGGAGCAGCGTCAA GGTATCTTGTAAAGCGTCTGGTTACGCCCTTTCAAACTACCTGATCGAATGGGTGAG GCAGGCTCCCGGCCAAGGCCTGGAATGGATGGGAGTTATCACCCCTGGGAGCGGCA CCATTAATTACGCCCAGAAATTTCAGGGACGAGTGACGATTACCGCCGACGAGTCC ACCAGTACTGCCTACATGGAGCTGTCCTCACTCCGCAGCGAGGACACGGCAGTTTAC TACTGCGCCCGGAGTCGATGGGACCCTCTTTACTATTATGCTCTGGAATACTGGGGC CAGGGAACGACCGTTACAGTGTCATCTGCTAGCACAAAAGGACCATCAGTCTTCCC ACTTGCTCCTTCATCTAAGAGCACAAGTGGTGGCACTGCAGCCCTTGGCTGCCTGGT GAAAGATTATTTCCCCGAACCTGTTACAGTTTCTTGGAACTCCGGTGCACTGACATC

CGGAGTACACACTTTCCCAGCTGTGCTGCAGAGCTCAGGACTGTATAGCCTGTCTTC
GGTGGTCACTGTTCCATCGTCGAGTCTTGGCACACAGACATATATTTGCAACGTCAA
TCACAAGCCCTCCAACACAAAAGTGGATAAGAAGGTCGAGCCCAAATCTTGTGACA
AGACCCATACGTGTCCTCCCTGTCCCGCCCCTGAACTGCTGGGAGGCCCTTCTGTGT
TCCTGTTCCCACCTAAGCCAAAGGACACTCTGATGATCAGCCGGACTCCCGAGGTTA
CCTGTGTGGTGGTGGATGTGTCTCATGAAGACCCTGAGGTTAAGTTCAATTGGTACG
TGGATGGCGTCGAGGTGCATAACGCAAAAACCAAGCCGAGAGAGGAGCAGTACaatA
GCACCTATAGAGTAGTGAGCGTCCTGACTGTCTTACATCAGGATTGGCTCAATGGTA
AAGAATATAAGTGCAAGGTAAGCAACAAGGCCCTACCCGCACCAATAGAGAAGAC
CATCTCCAAGGCGAAAGGTCAGCCCAGGGAGCCCCAGGTTTATACACTGCCTCCCTC
ACGCGACGAATTAACAAAGAATCAGGTGTCTCTCACCTGTCTCGTCAAGGGCTTTTA
CCCTTCCGACATCGCCGTGGAGTGGGAATCCAATGGCCAGCCTGAGAACAATTATA
AGACAACTCCCCCAGTCCTGGATTCAGATGGGTCGTTCTTTCTATATAGTAAGTTGA
CCGTGGATAAGTCTCGCTGGCAACAGGGGAACGTGTTCTCTTGCTCTGTTATGCATG
AAGCGCTGCACAATCATTATACCCAGAAGTCCCTGTCCCTGAGCCCCGGGAAG (SEQ
ID NO:20)

018 Fab Heavy Chain (018FabHC) polypeptide sequence in an IgGl framework. CDRs are underlined

QVQLVQSGAEVKKPGSSVKVSCKASGYALSNYLIEWVRQAPGQGLEWMGVITPGSGTI
NYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSRWDPLYYYALEYWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:l)

018 full length light chain (fl018LC) polypeptide sequence. CDRs are underlined

DIVMTQSPDS LAVSLGERAT INCRASESVD NYGIPFMNWY QQKPGQPPKL
LIYAASNRGS GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSEEVPL
TFGQGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV
QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV
THQGLSSPVT KSFNRGEC (SEQ ID NO:2)

This is also the light chain sequence for 020 and 029 IL-6 antagonists

018 full length light chain (018LC) nucleic acid sequence in an IgGl framework
GACATAGTGA TGACTCAAAG TCCGGACAGC CTGGCGGTGT CACTCGGCGA
ACGGGCAACT ATCAACTGCC GAGCCAGCGA GAGCGTCGAT AATTACGGCA
TCCCCTTCAT GAACTGGTAT CAGCAGAAGC CAGGACAGCC GCCCAAGCTG
CTTATCTACG CCGCTTCCAA CCGGGGATCA GGGGTGCCCG ATCGATTTAG
TGGAAGCGGT AGTGGGACCG ATTTCACACT GACCATCAGC TCCCTTCAGG
CCGAGGATGT GGCTGTCTAT TATTGTCAGC AATCCGAGGA AGTGCCGCTC
ACGTTTGGTC AGGGAACCAA ACTGGAGATC AAGCGGACCG TAGCGGCGCC
TAGTGTCTTC ATCTTCCCAC CCTCCGACGA ACAGCTGAAG TCTGGCACTG
CTTCCGTCGT GTGCCTGCTC AACAACTTTT ACCCTAGAGA GGCAAAAGTT
CAATGGAAAG TAGACAATGC CTTGCAGTCC GGGAACTCCC AGGAGTCTGT
CACAGAGCAG GATAGTAAGG ACTCAACCTA CAGCCTGTCC AGCACACTGA
CCCTCTCCAA AGCCGACTAC GAGAAGCACA AAGTGTACGC TTGCGAAGTT
ACGCATCAGG GGCTGTCCTC ACCCGTTACA AAAAGTTTTA ACAGAGGGGA GTGC
(SEQ ID NO:26)

019 Fab Heavy Chain (019FabHC, same sequence as 018FabHC except for A79V (bold/italic)
QVQLVQSGAE VKKPGSSVKV SCKASGYALS NYLIEWVRQA PGQGLEWMGV
ITPGSGTINY AQKFQGRVTI TADESTSTFY MELSSLRSED TAVYYCARSR
WDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPSSKST SGGTAALGCL
VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT
QTYICNVNHK PSNTKVDKKV EPKSC (SEQ ID NO:3)

019 VH (variable region/0 19HC)
QVQLVQSGAE VKKPGSSVKV SCKASGYALS NYLIEWVRQA PGQGLEWMGV
ITPGSGTINY AQKFQGRVTI TADESTSTFY MELSSLRSED TAVYYCARSR
WDPLYYYALE YWGQGTTVTV SS (SEQ ID NO:27)

The 019 antibody light chain (019LC) sequence (polypeptide and nucleic acid) is the same as the 018LC

CDR1 of 018HC (VH CDR1 018): GYALSNYLIE (SEQ ID NO:4)
CDR2 of 018HC (VH CDR2 018): VITPGSGTIN (SEQ ID NO:5)
CDR3 of 018HC (VH CDR3 018): SRWDPLYYYALEY (SEQ ID NO:6)
CDR1 of 018LC (VL CDR1): RASESVDNYGIPFMN (SEQ ID NO:7)
CDR2 of 018LC (VL CDR2): AASNRGS (SEQ ID NO: 8)
CDR3 of 018LC (VL CDR3): QQSEEVPLT (SEQ ID NO:9)
CDR1 of 019HC (VH CDR1 019): GYALSNYLIE (SEQ ID NO:4)
CDR2 of 019HC (VH CDR2 019): VITPGSGTIN (SEQ ID NO:5)
CDR3 of 019HC (VH CDR3 019): SRWDPLYYYALEY (SEQ ID NO:6)

Example 5: Epitope and structure mapping

Epitope mapping

Functional epitope mapping was performed on selected candidate IL-6 antagonists. It was found that a candidate antibody (murine 64 antibody) did not reduce binding of IL-6Ra to IL-6 in an ELISA indicating that the candidate antibody is not binding to site I. Additional experiments were conducted demonstrating that chimeric murine 64 antibody reduced binding of IL-6/IL-6Ra complex to gpl30 in an ELISA indicating that either Site II or Site III of IL-6 harbored the binding site for the antibody. It was also found that murine 64 antibody did not significantly block binding of a known site III binding antibody AH-65 (Immunotech, Marseille, France) to IL-6 indicating that the candidate antibody binds site II of IL-6. These data demonstrate that antibodies against site II can be generated and demonstrates a method of identifying such antibodies.

To further define the epitope, mutations in IL-6 were generated in yeast as fusions to 4m5.3 (Boder et al, 2000, Proc Natl Acad Sci USA 97, 10701-10705; Chao et al, 2006 , Nat Protoc 1, 755-768). The mutations expressed were in human IL-6 with the following single or double mutations: R24E/D27E, R30E, Y31E, D34R, S118RV121E, W157E, Q159E/T162P, K171E, and R179E. The expressed mutated IL-6 molecules were used in binding studies with 018 (Fab). Reduced affinity for 018 (Fab) was observed for R24E/K27E, Y31E, D34R, and SI 18RV121R, all of which are located in site II of IL-6. Accordingly, the invention described

herein includes an antibody that binds to at least one, two, three, four, five, or six of the amino acids at position 24, 27, 31, 34, 118, and 121 of human IL-6 or the equivalent site in an IL-6.

Structural definition of a site II epitope

The following distances were calculated to structurally define site II. The calculations are based on the IL-6/IL-6a/gpl30 hexameric crystal structure, PDB 1P9M (Boulanger et al, 2003, Science 300: 2101-2104). Helix 1 of IL-6 runs between site I and site II resulting in certain residues that fall close to site II but have side chains that point toward site I, e.g., R30. D2 and D3 refer to extracellular domains of IL-6Ra.

The following amino acids of IL-6 were determined to fall within 5 **A** of gpl30-D2-D3: L19, R24, K27, Q28, R30, Y31, D34, E 110, Q1 l I, R 113, A 114, M 117, S 118, V121, Q124, F125, and K128

The following amino acids were determined to fall within 7 **A** of gpl30-D2-D3: L I9, E23, R24, 125, K27, Q28, 129, R30, Y31, D34, K41, Q102, E109, E l 10, Q l 11, A l 12, R l 13, A 114, V 115, Q 116, M l 17, S 118, K120, V121, L122, Q124, F125, and K128.

Accordingly, a molecule, e.g., an antibody or fragment thereof that can bind one or more of the IL-6 amino acids falling within 5 **A** or 7 **A** of site II can be an IL-6a.

The sequence of human IL-6 is provided below for reference (underlined sequence is the leader sequence). Amino acids within 7 **A** of gpl30-D2-D3 are in italics. The amino acid numbering, e.g., mutations used to define epitopes, is without the leader sequence:

Human IL-6

MNSFSTSAFGPVAFSLGLLLVLPAAFPA PVPPGEDSKDVAAPHRQPZTSS *EiRIDKQIi?YILD* GISALR*K*ETCNKSNMCESSKEALAENNLNLPKMAEKD GCFQ SGFNEETCL VKIITGLLEF EVYLEYL*Q*NRFESS*EEQARAVQMS*T*KVLIQF*LQ*K*KAKNLDAITTPDPTTNASLLTKLQAQ NQWLQDMTTHLILRSFKEFLQSSLRALRQM (SEQ ID NO:21)

Experiments were conducted testing the Fab fragment of the h64-1.4 humanized antibody and demonstrated that it was able to block both cis and trans IL-6 signaling, which is due to site II targeting. The potency of the Fab fragment was unchanged in the presence of soluble IL-6 receptor (sIL-6R). This is in contrast to an anti-IL-6R IgG that had decreased potency in the presence of sIL6R, and that blocks cis signaling only.

These experiments demonstrate that an antibody or fragment of the antibody such as a Fab fragment that targets site II can be used to inhibit both cis and trans signaling of 11-6.

Example 6: Primate studies

Because non-primate activities can differ greatly from those of primates, candidate IL-6 antagonists are typically further assessed for PK and other parameters using non-human primates. Human IL-6 differs from cynomolgus monkey and rhesus monkey IL-6 at seven sites, one of which is in site II (amino acid 28) and is the same at site II in African green monkey IL-6. This appears to decrease binding of an antibody comprising 018 sequences by only about 3-4 fold. The ability to bind to a non-human primate IL-6 is a useful feature of an IL-6 antagonist, facilitating development of the candidate as a drug, e.g., by enabling testing such as toxicology testing in non-human primates.

As with most IL-6 antibodies, anti-IL-6 antibodies described herein did not cross-react to rodent, rabbit, or canine IL-6 due to low sequence homology. However, in affinity studies, it was found that 018 Fab binds cynomolgus monkey and African green monkey IL-6 with approximately human affinity (Table 2).

Table 2: Monovalent affinity (018 Fab) for various IL-6 of various species

Species	K_D
Human	200 pM
African Green Monkey	280 pM
Cynomolgus monkey	840 pM
Dog	> 1 μM
Mouse	> 1 μM
Rabbit	> 1 μM
Rat	> 1 μM

These data further demonstrate the ability of an IL-6a as described herein to specifically bind and the ability to develop a molecule having features permitting testing, e.g., for toxicology and reproductive studies, in a suitable animal.

Example 7: Increasing expression of an IL-6a

To increase expression of 018 Fab and 019 Fab polypeptides, constructs were made introducing five additional amino acids (DKTHT (SEQ ID NO: 30)) to the heavy chain in the CHI /hinge region using methods known in the art. The sequence of the altered 018Fab heavy chain is shown below as SEQ ID NO:24 . The altered 018 sequence is referred to herein as 020 and the altered 019 sequence is referred to herein as 021. The 020 molecule (the 020Fab heavy chain and the 018Fab light chain) had improved expression compared to the parent Fab that had 018Fab heavy and 018Fab light chains. The 019 molecule exhibited no significant affinity difference compared to the 020 molecule. Expression of both 020 and 019 was increased by about two fold, respectively, and the affinities were not affected by the alteration.

020 Heavy chain (Fab with DKTHT (SEQ ID NO: 30) at the carboxy terminus))
QVQLVQSGAE VKKPGSSVKV SCKASGYALS NYLIEWVRQA PGQGLEWMGV ITPGSGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPSSKST SGGTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT QTYICNVNHK PSNTKVDKKV EPKSCDKTHT (SEQ ID NO:24)

IL-6 antagonism using the 020Fab was measured in HEK-Blue™ IL-6 reporter cells (InvivoGen, San Diego, CA). Cells were incubated in a mixture of 10 pM IL-6 and varying concentrations of either 020 or IL-6Ra antibody (Cell Sciences, Canton, MA), with or without 50 nM IL-6Ra. After 20-24 hours of incubation, 20 μL of cell culture supernatant was mixed with 180 μL of QuantiBlue™ (InvivoGen) substrate and incubated for one hour; the absorbance was then measured at 655 nm. Fig. 3A and Fig. 3B show data from these experiments, demonstrating the ability of 020 to inhibit IL-6 activity in the presence or absence of IL-6R.

Example 8: IgG2 IL-6 antibodies

018 was reformatted into a human IgG2 isotype framework to reduce FcyR binding and reduce ADCC compared to the IgGl formatted antibody using methods known in the art. In

addition, reformatting 018 to a full-length format, e.g., an IgG2, is expected to decrease the rate of clearance from the vitreous due to the larger size of the molecule.

Construction/purification of anti-IL6 IgG2 antibodies

To construct human IgG2 antibodies using anti-IL-6 sequences described *supra*, a human IgG2 constant domain was PCR amplified from cDNA with Nhel and Mlul restriction sites at the N- and C-terminal ends, respectively. The PCR product was purified, digested with Nhel and Mlul restriction enzymes, and then ligated into pTT5 vector containing anti-IL6 variable domain, i.e., SEQ ID NO:l (see above). This yielded a full-length IgG2 heavy chain sequence. Plasmids containing the full-length light chain containing the 018 sequence were used to provide light chain.

To further reduce FcRn binding and thereby reduce recycling of the IL-6a, point mutations were made in the heavy chain. The mutations were made by QuikChange® mutagenesis (Agilent Technologies, Santa Clara, CA). The heavy and light chain plasmids were co-transfected using poly(ethylenimine) (PEI) into 100 mL transient cultures of HEK293-6E cells and cultured to allow expression for about five days. This generated antibodies containing an anti-IL-6 site II binding moiety and IgG2 structure. Such structures containing 018 CDRs are termed herein 018IgG2 or 029. The point mutations were made at residues 1253

The IgG2 molecule was well expressed and blocks IL-6 in cellular assays with slightly improved potency compared to the 020Fab.

029 mature sequences (CDRs underlined)

029 Heavy chain

QVQLVQSGAE VKKPGSSVKV SCKASGYALS NYLIEWVRQA PGQGLEWMGV ITPGSGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD

SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK (SEQ ID NO:l l)

029 Light chain

DIVMTQSPDS LAVSLGERAT INCRASESVD NYGIPFMNWY QQKPGQPPKL LIYAASNRGS GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSEEVPL TFGQGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC (SEQ ID NO: 12)

Altered FcRn binding

IL-6 can have certain positive systemic effects. It is therefore an advantage to engineer an IL-6a that has good retention in the vitreous but has a limited systemic half-life. The reduction or elimination of FcRn binding should reduce systemic accumulation of any drug that escapes into circulation, thereby improving safety of an IL-6a.

Accordingly, because FcRn mediated trafficking may increase the efflux of antibodies from the eye, the 020 IgG2 was further modified to ablate FcRn binding by introducing Fc mutations at residues 1254, H31 1, or H436 (See SEQ ID NO:23) numbering according to Martin et al, Molecular Cell, 7:4, 867-877 (2001)). The mutated sites are shown in boldface in SEQ ID NO:23; 1254 was mutated to A or R, H311 was mutated to A or E, H311 was mutated to N with D 313 mutated to T, and H436 was mutated to A (numbering starts after the leader sequence, which is underlined in SEQ ID NO:23. IL-6 antagonists containing such sequences are termed 018IgG2m.

Anti-IL-6 heavy chain (IgG2) (regular font: VH; italic font: CH) (without leader sequence) showing mutation sites (boldface)

QVQLVQSGAE VKKPGSSVKV SCKASGYALS NYLIEWVRQA PGQGLEWMGV ITPGSGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV *SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVWDVSHE*

DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIA V EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK (SEQ ID NO:23)

Anti-IL-6 heavy chain (IgG2) (regular font: VH; italic font: CH) with leader sequence (underlined) showing mutation sites (boldface)
MDWTWRILFLVAAATGAHSQVQLVQSGAE VKKPGSSVKV SCKASGYALS NYLIEWVRQA PGQGLEWMGV ITPGSGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV *SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVWDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK* (SEQ ID NO:28)

Accordingly, some embodiments include an antibody having the heavy chain sequence depicted in SEQ ID NO:23 with mutations at 1254 (e.g., A or R), H311 (mutated to A or E), H436 (mutated to A), or D313 (mutated to T) with H311 mutated to N.

SEQ ID NO:25 therefore provides a sequence that, when mutated at 1133 (e.g., I133A or I133R), H190 (e.g., H190A or H190E), H315 (e.g., H315A), or D192 with H190 (e.g., D192T with H190N) can be used in an antibody, fragment, or derivative thereof to produce a polypeptide having reduced Fc binding at low pH, e.g., pH 5.5 or lysosomal pH and/or a polypeptide having reduced systemic half-life compared to a parent or other reference molecule that does not include the sequence.

SASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVWDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV

KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK (SEQ ID NO:25)

Anti-IL-6 light chain (IgG2) (regular font: VK; italic font: CK)

DIVMTQSPDSLAVSLGERATINCRASESVDNYGIPFMNWYQQKPGQPPKLLIYAASNRG SGVPDPvFSGSGSGTDFTLTISSLQAEDVAVYYCQQSEEVPLTFGQGTKLEIKi *RTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC* (SEQ ID NO:22)

Example 9: Formulation stability

The stability of the anti-IL-6/IgGl Fab fragment (containing the IgGlCHl domain) was tested by determining the T_m initially in PBS then in a range of buffers and excipients using differential scanning fluorimetry. It was found that citrate buffer, pH 5.5 increased the T_m to more than 80°C. Accordingly, in some embodiments, an IL-6a is provided in citrate buffer and in some cases has a T_m of at least 80°C.

Aggregation was tested using SEC-MALS and no aggregation was observed at 20 mg/ml in phosphate buffered saline (PBS).

Example 10: pH sensitive antibodies for enhanced PK

IL-6 can have certain positive systemic effects. It is therefore an advantage to engineer an IL-6a that has good retention in the vitreous but has a limited systemic half-life. The reduction or elimination of FcRn binding should reduce systemic accumulation of any drug that escapes into circulation, thereby improving safety of an IL-6a. Accordingly, because FcRn mediated trafficking may increase the efflux of antibodies from the eye, the 020 IgG2 was further modified to ablate FcRn binding by introducing Fc mutations at residues 1253, H310, or H435 (numbering according to Martin et al. (Molecular Cell, 7:4,867-877 (2001))). Such antibodies are referred to herein as IL-6pH antibodies or anti-IL-6pH and are further described below.

Generation of antibodies with pH sensitive binding

The pKa of histidine is about 6.0 and histidines inserted at binding interfaces can disrupt binding upon side-chain protonation at low pH. Using an anti-IL-6 site II targeted antibody as described herein, a library was generated containing histidine-rich variants of CDRs from 018

and the library was screened for pH-sensitive binding using yeast display. The library generated was a combinatorial library with CDRs encoded by degenerate codons such that each residue is either a wild-type residue (i.e., the same as in the parental antibody) or a histidine residue. The screening was performed by alternating sorting for high binding at physiological pH (7.4) and low binding at endosomal pH (5.5).

A yeast-selected mutant was identified that had relatively high binding at pH 7.4 (monovalent Kd of 407 pM for the mutant compared to 192 pM for the parent molecule) and relatively low binding at pH 5.5 (monovalent Kd of 2.362 nM for the mutant compared to 195 pM for the parent). This constitutes an approximately 5.8 fold change in the affinity at pH 5.5. This mutant contained multiple histidine mutations in the light chain CDR1. Thus, the mutant demonstrated similar binding to the parent molecule at pH 7.4, and a significant loss of affinity at pH 5.5. This observation was verified using ELISA, FACS, and SPR analysis by methods known in the art.

These data demonstrate that an IL-6a that is based on an antibody can be created that has the features of an anti-IL-6 targeting site II of IL-6 that can be used to inhibit both cis and trans activity of IL-6, and have increased PK compared to a parent antibody or other antibody having a wild type Fc domain effected at least in part by altered binding at pH 5.5.

Example 11: Efficacy of local IL-6 blockade in mouse laser choroidal neovascularization (CNV) model

To determine whether local IL-6 blockade could be effective for treating eye disease, e.g., diabetic macular edema (DME) or wet AMD, a monoclonal anti-IL-6 antibody was locally administered in a model system for choroidal neovascularization. The laser-induced CNV model as described in Saishin et al. Journal of Cellular Physiology, 195:241-248 (2003) was employed in this Example. A laser-induced CNV model reproduces many of the pathologic processes underlying diabetic macular edema (DME), including inflammation and angiogenesis.

A monoclonal anti-mouse IL-6 antibody (MP5-20F3, which is a rat IgGl isotype antibody purchased from Bio X Cell, catalog number BE0046) was administered to the test group by intravitreal (IVT) injection. Controls received intravitreal injection of VEGF trap or intravitreal injection of an anti-HRP isotype control antibody (a rat IgGl against horseradish peroxidase, clone HRPN, purchased from BioXCell; catalog number BE0088). For all antibody

groups, 20 μg of protein in a 1μL volume was injected into the test eye, while the contralateral eye was left untreated as a further control.

Mice were euthalized on day 7 after laser and choroidal flat mounts were stained with Griffonia Simplicifolia (GSA) lectin to measure the lesion area. Fig. 4 shows the results. The anti-IL-6 antibody treated group showed a statistically significant reduction in neovascularization compared to the control antibody treated group ($p<0.05$). On average the anti-IL-6 antibody treated group also showed reduced neovascularization compared with the anti-VEGF positive control.

These data demonstrate that an IL-6a, e.g., a monoclonal anti-IL-6 antibody, administered IVT can significantly reduce neovascularization in a mouse CNV model. The results further suggest that an anti-IL-6 antibody can produce a reduction in neovascularization at least as great, and possibly greater, than an anti-VEGF antibody. These data indicate that local inhibition of IL-6 is useful for treating eye diseases such as diseases involving vascular leakage, e.g., wet AMD or macular edema, e.g., diabetic macular edema.

Example 12: Development of an improved IL-6 antibody

Variants of the EBI-029 antibody were generated. To better characterize the contribution of mutations A28V, S30P, 15 IT, and S55G, specific combinations were introduced into the wild-type EBI-029 Fab display vector and binding measured. The results are shown in Fig. 5. After overnight competition with 2 μM IL-6, all mutants had significantly higher levels of biotinylated IL-6 remaining on their cell surface relative to display compared to the wild-type EBI-029 Fab. The rank order of binding from highest to lowest affinity was A28V/S30P/I51T/S55G > A28V/I5 1T/S55G > S30P/I5 1T/S55G > 15 1T/S55G > wt. The quadruple mutation A28V/S30P/I51T/S55G is also referred to herein as EBI-030.

Sequences of EBI-030 are shown below.

030 CDR sequences:
CDR1 of 030HC (VH CDR1 030): GYVLPNYLIE (SEQ ID NO:31)
CDR2 of 030HC (VH CDR2 030): VTTPGGGTIN (SEQ ID NO:32)
CDR3 of 030HC (VH CDR3 030): SRWDPLYYYALEY (SEQ ID NO:33)

CDR1 of 030LC (VL CDR1 030): RASESVDNYGIPFMN (SEQ ID NO:34)
CDR2 of 030LC (VL CDR2 030): AASNRGS (SEQ ID NO:35)
CDR3 of 030LC (VL CDR3 030): QQSEEVPLT (SEQ ID NO:36)

030 heavy chain variable region sequence (mutations relative to 029 shown in bold):
QVQLVQSGAE VKKPGSSVKV SCKASGYVLP NYLIEWVRQA PGQGLEWMGV TTPGGGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV SS (SEQ ID NO:37)

030 light chain variable region sequence:
DIVMTQSPDSLAVSLGERATINC RASESVDNYGIPFMN WYQQKPGQPPKLLIY AASNRG SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYY CQQSEEVPLT FGQGTKLEIKRTV (SEQ ID NO:38)

030 Fab (IgGl) heavy chain polypeptide sequence (CDRs underlined, mutations relative to 029 shown in bold):
QVQLVQSGAE VKKPGSSVKV SCKASGYVLP NYLIEWVRQA PGQGLEWMGV TTPGGGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPSSKST SGGTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT QTYICNVNHK PSNTKVDKKV EPKSCDKTHT (SEQ ID NO:39)

In embodiments, the DKTHT sequence (SEQ ID NO:30) at the carboxy terminus of SEQ ID NO:39 is not included in the Fab sequence.

030 Fab heavy chain nucleic acid sequence:

CAAGTGCAGCTGGTGCAGTCAGGGGCCGAGGTTAAGAAGCCAGGGAGCAGCGTCAA
GGTATCTTGTAAAGCGTCTGGTTACGTCCTTCCAAACTACCTGATCGAATGGGTGAG
GCAGGCTCCCGGCCAAGGCCTGGAATGGATGGGAGTTACCACCCCTGGGGGCGGCA
CCATTAATTACGCCCAGAAATTTCAGGGACGAGTGACGATTACCGCCGACGAGTCC

ACCAGTACTGCCTACATGGAGCTGTCCTCACTCCGCAGCGAGGACACGGCAGTTTAC
TACTGCGCCCGGAGTCGATGGGACCCTCTTTACTATTATGCTCTGGAATACTGGGGC
CAGGGAACGACCGTTACAGTGTCATCTGCTAGCACAAAAGGACCATCAGTCTTCCC
ACTTGCTCCTTCATCTAAGAGCACAAGTGGTGGCACTGCAGCCCTTGGCTGCCTGGT
GAAAGATTATTTCCCCGAACCTGTTACAGTTTCTTGGAACTCCGGTGCACTGACATC
CGGAGTACACACTTTCCCAGCTGTGCTGCAGAGCTCAGGACTGTATAGCCTGTCTTC
GGTGGTCACTGTTCCATCGTCGAGTCTTGGCACACAGACATATATTTGCAACGTCAA
TCACAAGCCCTCCAACACAAAAGTGGATAAGAAGGTCGAGCCCAAATCTTGTGACA
AAACACACACA(SEQ ID NO:40)

030 can also be produced as an IgG2 Fab heavy chain polypeptide sequence:
QVQLVQSGAEVKKPGSSVKVSCKASGYVLPNYLIEWVRQAPGQGLEWMGVTTPGGGTI
NYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSRWDPLYYYALEYWGQGT
TVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERK
(SEQ ID NO:68)

Example 13: Expression and purification of variant Fab fragments

VH domain inserts containing the following mutant combinations, A28V/I51T/S55G, S30P/I51T/S55G, and A28V/S30P/I51T/S55G (EBI-030), were generated from the yeast display vectors by double digest with BamHI-HF/Nhel-HF. Inserts were purified by 1% agarose gel electrophoresis and ligated into a pTT5 derived mammalian expression vector containing a leader sequence, human IgGl CHI domain, and C-terminal His tag. Transformants were selected on LB-Amp, miniprepped, and the inserts confirmed by sequencing. Transient transfections were performed in HEK-6E cells (Canadian Research Council) for each mutant Fab heavy chain paired with the wild-type EBI-029 light chain (disclosed herein as SEQ ID NO: 12) using PEI as a transfection reagent. The wild-type EBI-029 Fab was also expressed as a control (the wild-type Fab heavy chain is disclosed herein as SEQ ID NO:24). Supernatants were harvested after 5 days and the expressed Fabs purified by affinity chromatography using Ni-NTA agarose (Life Technologies). Purified protein was buffer exchanged into PBS, pH 7.4 by several

rounds of concentration/dilution and protein concentration and purity determined by Absorbance 280 and SDS-PAGE.

Example 14: Variant antibodies showed improved binding as assessed using surface plasmon resonance

Affinities of the variant 029 Fab molecules for IL-6 were measured by Surface Plasmon Resonance (SPR) on a Reichert SR7000Dc Spectrometer. Human IL-6 at 20 μg/mL in 10 mM sodium acetate, pH 4.5 was immobilized on a 500-kDa carboxymethyl dextran chip via standard amine coupling. Serial dilutions of each Fab molecule in 10 mM HEPES, 150 mM NaCl, pH 7.3 were injected at 25°C with a 25 μL/min flow rate. After 4 minutes, loading was stopped and dissociation measured by flowing running buffer (10 mM HEPES, 150 mM NaCl, pH 7.3) for 5 minutes. Sensogram traces fit poorly to a 1:1 binding model, potentially due to mixed orientations of IL-6 on the chip or non-specific antibody binding. Instead, curves were fit to a 2 species (low affinity and high affinity species, labeled "low affinity" and "high affinity" in table 3) fit using TraceDrawer software where kal, kdl, and KDl are the association rate, dissociation rate, and equilibrium binding constant for the low affinity species, and ka2, kd2, and KD2 are the association rate, dissociation rate, and equilibrium binding constant for the high affinity species. All mutant Fabs had significantly slower dissociation compared to the wt EBI-029 Fab with the following rank order of highest to lowest affinity - A28V/S30P/I51T/S55G (EBI-030) > S30P/I51T/S55G > A28V/I51T/S55G > WT (EBI-029).

Table 3: SPR results for mutant antibodies

Fab	ka1 (*e4)	kd1 (*e-2)	KD1 (nM)	ka2 (*e3)	kd2 (*e-4)	KD2 (nM)
WT	5.43	6.08	11.1	2.94	4.27	1.45
A28V/I51T/S55G	8.06	2.91	3.6	3.65	1.45	0.40
S30P/I51T/S55G	7.18	2.18	3.04	3.29	0.95	0.29
A28V/S30P/I51T/S55G	7.95	2.70	3.39	3.25	0.66	0.20
	Low affinity			High affinity		

Example 15: Variant antibodies showed improved antagonistic potency in HEK-Blue™ IL6 reporter cells

The HEK-Blue™ IL6 reporter cell line (Invivogen) was used to compare the potency of IL6 signaling inhibition between the different mutant EBI-029 Fab fragments. HEK-Blue™ IL6 cells are a modified HEK293 line stably expressing the IL-6R gene and containing a secreted alkaline phosphatase reporter gene under control of the IFNβ minimal promoter fused to four STAT3 binding sites. To measure IL6 antagonism, 10 μL of 400 pM human IL-6 (R&D Systems 206-IL-OlO/CF) was mixed with 10 μL of each Fab variant at a range of concentrations in a 96 well plate and incubated at RT for 30 minutes. HEK-Blue™ IL6 cells in log phase were trypsinized and resuspended in assay media (DMEM, 4.5 g/1 glucose, 10% Heat inactivated FBS, 2 mM L-glutamine, Pen-Step) at 280,000 cells/mL. 180 μL of cell suspension was added to each well of IL-6/Fab mixtures to bring the final IL-6 concentration to 20 pM. The cells were incubated at 37°C/5% $C0_2$ for 20 hours. 20 μL of supernatant from each well was then mixed with 180 μL of Quanti-Blue™ reagent (Invivogen) and incubated at 37°C for 40 minutes before measuring absorbance at 650 nM on a SpectraMax M5 plate reader. The background signal from wells with no IL-6 was subtracted and then divided by IL-6 treated cells with no inhibitor to derive a fractional signaling value. All mutants showed significantly greater potency compared to the wt EBI-029 Fab with the rank order of antagonistic potency as follows:

A28V/S30P/I51T/S55G (EBI-030) > A28V/I51T/S55G > S30P/I51T/S55G > WT (EBI-029). These results are shown in Fig. 6.

Example 16: Variant antibodies showed improved antagonistic potency in T1165 proliferation assay

Tl 165.85.2.1 cells (R&D Systems) are a murine plasmacytoma cell line that proliferates in response to mouse, rat, or human IL-6. To measure antagonism from the EBI-029 Fab mutants, 25 μL of 2 ng/mL human IL-6 (R&D Systems 206-IL-OlO/CF) was mixed with 25 μL of each Fab variant at a range of concentrations in a 96 well plate and incubated at RT for 30 minutes. Tl 165 cells in log phase were pelleted and resuspended in assay media (90% RPMI 1640, 10% FBS, 2 mM L-glutamine, Pen-Strep) at 2x105 cells/mL. 50 μL of cell suspension was added to each well of IL-6/Fab mixtures to bring the final IL-6 concentration to 0.5 ng/mL. The cells were incubated at 37oC/5%> C02 for 72 hours. 100 μï¸ of Cell-Titer Glo® reagent (Promega) was added to each well and incubated at RT for 10 minutes. Luminescence was measured on a SpectraMax M5 plate reader. All mutants showed significantly greater potency compared to the wt EBI-029 Fab with no measurable IL-6 signaling over the range of Fab concentrations tested (see Fig. 7).

Example 17: Drug like properties comparison of variant antibodies

Thermal stability of each Fab variant was determined by differential scanning fluorimetry (DSF). 2 μï¸ of protein at 2.5 or 5 mg/mL was mixed with 18 μL PBS and 2 μï¸ of 50x Sypro Orange in a BioRad 96 well PCR plate. The plate was run in a BioRad CFX96 RT-PCR System with a linear temperature increase from 25°C and 95°C and fluorescence measured over time. The T_m was calculated as the lowest point of the first derivative of the melt curve. All variants had measured T_m values between 76 and 78°C, consistent with the measured T_m of the wt EBI-029 Fab at 76°C.

To measure aggregation, samples were assessed by SEC-MALS using an Agilent 1260 HPLC combined with a Wyatt miniDawn TREOS light scattering instrument and Wyatt Optilab rEX refractive index instrument. 20 - 100 μg of protein was injected and run at a flow rate of 1 mL/min. All variants had molecular weights between 45000 and 52000 Da as measured by light scattering, consistent with the wild-type EBI-029 Fab.

These results indicate that EBI-030 behaves similarly well compared with EBI-029 in terms of its drug like properties.

Example 18: Production of full length EBI-029 and EBI-030 IgG2 antibodies and IgG2 antibodies with mutant Fc domains

Reformatting EBI-029 and EBI-030 to IgG2 and mutant Fc IgG2

The heavy chain variable domains of EBI-029 and EBI-030 including the leader sequence (MDWTWRILFLVAAATGAHS; SEQ ID NO:49) were PCR amplified from the Fab vectors using primers that introduced an N-terminal EcoRI site and C-terminal Nhel site. PCR products were purified on a 1% agarose gel and double digested with EcoRI-HF & Nhel-HF. pTT5 based backbone vectors containing the wild-type IgG2 heavy chain sequence or a variant IgG2 domain with an H311A mutation (H311 corresponds to the numbering in SEQ ID NO:41; this corresponds to H310 in the numbering provided in Martin et al, Molecular Cell, 7:4, 867-877 (2001)) were similarly digested EcoRI-FH/Nhel-HF and purified on a 1% agarose gel. Inserts were ligated into the digested backbone using Quikligase enzyme (New England Biolabs), transformed in TOP 10 cells (Life Technologies), and selected on LB-Amp. Clones were miniprepped and sequenced to confirm the insert. The H311A mutation was selected to reduce Fc binding affinity for FcRn in order to reduce systemic accumulation of molecules that escape from the ocular tissue.

Expression and purification of IgG2 variants by transient transfection

EBI-029 IgG2, EBI-029 IgG2-H311A, EBI-030 IgG2, and EBI-030 IgG2-H311A were expressed by transient transfection in HEK-6E cells. pTT5 vectors containing each heavy chain were cotransfected with the EBI-029 LC plasmid using PEI as a transfection reagent. Supernatants were harvested after 5 days and the expressed IgG2 molecules purified by affinity chromatography using Protein-A agarose. Purified protein was buffer exchanged into PBS, pH 7.4 by several rounds of concentration/dilution and protein concentration and purity determined by Absorbance 280 and SDS-PAGE.

CHO stable pool production

Stable CHO pools producing EBI-029 IgG2, EBI-030 IgG2, or EBI-030 IgG2-H31 1A were generated using the Freedom CHO-S kit (Life Technologies) according to manufacturer's instructions. In short, each heavy chain was cloned by standard digestion/ligation into the pCHO 1.0 vector in combination with the EBI-029 LC. Constructs were transfected into CHO-S cells using Freestyle MAX reagent and stable pools selected with increasing concentrations of Puromycin and MTX. Following two rounds of selection, pools were screened for antibody production by analytical Protein-A chromatography and the highest producers were selected for scale-up and subcloning.

Sequences are presented below.

030 Heavy chain polypeptide sequence (in IgG2 framework, CDRs underlined): QVQLVQSGAE VKKPGSSVKV SCKASGYVLP NYLIEWVRQA PGQGLEWMGV TTPGGGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK (SEQ ID NO:41)

030 light chain polypeptide sequence (in IgG2 framework, CDRs underlined): DIVMTQSPDS LAVSLGERAT INCRASESVD NYGIPFMNWY QQKPGQPPKL LIYAASNRGS GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSEEVPL TFGQGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC (SEQ ID NO:42)

030 heavy chain nucleic acid sequence:

CAAGTGCAGCTGGTGCAGTCAGGGGCCGAGGTTAAGAAGCCAGGGAGCAGCGTCAA
GGTATCTTGTAAAGCGTCTGGTTACGTCCTTCCAAACTACCTGATCGAATGGGTGAG
GCAGGCTCCCGGCCAAGGCCTGGAATGGATGGGAGTTACCACCCCTGGGGGCGGCA
CCATTAATTACGCCCAGAAATTTCAGGGACGAGTGACGATTACCGCCGACGAGTCC
ACCAGTACTGCCTACATGGAGCTGTCCTCACTCCGCAGCGAGGACACGGCAGTTTAC
TACTGCGCCCGGAGTCGATGGGACCCTCTTTACTATTATGCTCTGGAATACTGGGGC
CAGGGAACGACCGTTACAGTGTCATCTGCTAGCACCAAGGGCCCATCGGTCTTCCCC
CTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGT
CAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCTCTGACCA
GCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCA
GCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAG
ATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTC
GAGTGCCCACCGTGCCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCC
CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGT
GGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTC
CGTGTGGTCAGCGTCCTCACCGTCGTGCACCAGGACTGGCTGAACGGCAAGGAGTA
CAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGAGAAAACCATCTCCA
AAACCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAG
GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAG
CGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG
GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGC
TCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA SEQ ID
NO:43

030 light chain nucleic acid sequence:

GACATAGTGATGACTCAAAGTCCGGACAGCCTGGCGGTGTCACTCGGCGAACGGGC
AACTATCAACTGCCGAGCCAGCGAGAGCGTCGATAATTACGGCATCCCCTTCATGA
ACTGGTATCAGCAGAAGCCAGGACAGCCGCCCAAGCTGCTTATCTACGCCGCTTCCA

ACCGGGGATCAGGGGTGCCCGATCGATTTAGTGGAAGCGGTAGTGGGACCGATTTC
ACACTGACCATCAGCTCCCTTCAGGCCGAGGATGTGGCTGTCTATTATTGTCAGCAA
TCCGAGGAAGTGCCGCTCACGTTTGGTCAGGGAACCAAACTGGAGATCAAGCGGAC
CGTAGCGGCGCCTAGTGTCTTCATCTTCCCACCCTCCGACGAACAGCTGAAGTCTGG
CACTGCTTCCGTCGTGTGCCTGCTCAACAACTTTTACCCTAGAGAGGCAAAAGTTCA
ATGGAAAGTAGACAATGCCTTGCAGTCCGGGAACTCCCAGGAGTCTGTCACAGAGC
AGGATAGTAAGGACTCAACCTACAGCCTGTCCAGCACACTGACCCTCTCCAAAGCC
GACTACGAGAAGCACAAAGTGTACGCTTGCGAAGTTACGCATCAGGGGCTGTCCTC
ACCCGTTACAAAAAGTTTTAACAGAGGGGAGTGCSEQ ID NO:44

030 Heavy chain polypeptide sequence with the H311A mutation (311A is boldface and CDRs are underlined), also referred to herein as the **031** heavy chain polypeptide sequence:

QVQLVQSGAE VKKPGSSVKV SCKASGYVLP NYLIEWVRQA PGQGLEWMGV TTPGGGTINY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARSR WDPLYYYALE YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV AQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK (SEQ ID NO:47)

031 heavy chain nucleic acid sequence:
CAAGTGCAGCTGGTGCAGTCAGGGGCCGAGGTTAAGAAGCCAGGGAGCAGCGTCAA
GGTATCTTGTAAAGCGTCTGGTTACGTCCTTCCAAACTACCTGATCGAATGGGTGAG
GCAGGCTCCCGGCCAAGGCCTGGAATGGATGGGAGTTACCACCCCTGGGGGCGGCA
CCATTAATTACGCCCAGAAATTTCAGGGACGAGTGACGATTACCGCCGACGAGTCC
ACCAGTACTGCCTACATGGAGCTGTCCTCACTCCGCAGCGAGGACACGGCAGTTTAC
TACTGCGCCCGGAGTCGATGGGACCCTCTTTACTATTATGCTCTGGAATACTGGGGC

CAGGGAACGACCGTTACAGTGTCATCTGCTAGCACCAAGGGCCCATCGGTCTTCCCC
CTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGT
CAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCTCTGACCA
GCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCA
GCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAG
ATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTC
GAGTGCCCACCGTGCCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCC
CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGT
GGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTC
CGTGTGGTCAGCGTCCTCACCGTCGTGGCCCAGGACTGGCTGAACGGCAAGGAGTA
CAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGAGAAAACCATCTCCA
AAACCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAG
GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAG
CGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG
GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGC
TCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID
NO:48)

Example 19: EBI-030 vs. EBI-029 IgG2 potency comparison in HEK-Blue-IL6 assay

The HEK-Blue™ IL6 reporter cell line (Invivogen) was used to compare the potency of IL6 signaling inhibition between EBI-029 and EBI-030 IgG2 antibodies. Three protein preps purified from HEK-6E cells were compared - EBI-029 IgG2, EBI-030 IgG2, and EBI-030 IgG2-H311A (also referred to as 031 or EBI-031), along with a prep of EBI-030 IgG2 produced in a stable CHO pool. Additionally, Tocilizumab, an approved anti-IL6R antibody, was included as a control. To measure IL6 antagonism, human IL-6 (R&D Systems 206-IL-OlO/CF) at 400 pM was mixed with varying concentrations of each antibody in a 96 well plate and incubated at RT for 30 minutes. HEK-Blue™ IL6 cells in log phase were trypsinized and resuspended in assay media (DMEM, 4.5 g/l glucose, 10% Heat inactivated FBS, 2 mM L-glutamine, Pen-Step) at 280,000 cells/mL. 180 μL of cell suspension was added to each well of IL-6/Fab mixtures to

bring the final IL-6 concentration to 20 pM. The cells were incubated at 37°C/5% $C0_2$ for 20 hours. 20 μL of supernatant from each well was then mixed with 180 μL of Quanti-BlueTM reagent (Invivogen) and incubated at 37°C for 40 minutes before measuring absorbance at 650 nM on a SpectraMax M5 plate reader.

The results are shown in Fig. 8 and in Table 5. EBI-030 (including EBI-030 produced in HEK cells with or without the H311A mutation and EBI-030 produced in CHO cells) showed greatly improved potency (about a 50 fold decrease in IC50 and >100 fold decrease in IC90) compared with EBI-029. The increase in potency was greater than the increase in affinity measured by SPR.

Table 5: IC50 and IC90 values

	IC50 (pM)	IC90 (pM)
EBI-029	47	4350
EBI-030	0.9	1.1
EBI-030 CHO	1.4	11
EBI-030-H311A	0.6	12.4
Tocilizumab	1490	23700

EBI-031 (also referred to herein as EBI-030 IgG2-H311A) had an IC50 more than 75 fold less than that of EBI-029 and an IC90 about 350 fold less than that of EBI-029. EBI-030 produced in HEK cells had an IC50 more than 50 fold less than that of EBI-029 and an IC90 approximately 4000 fold less than that of EBI-029.

Example 20: Modeling analysis of increased potency on duration of vitreal IL-6 blockade

The effect of increased potency on the extent and duration of IL-6 blockade following intravitreal administration was simulated using a pharmacokinetic model (Fig. 9). Differential equations describing changes in free antibody (A), free IL-6 (IL), and the antibody/IL-6 complex (AIL) were defined as follows:

d/dt(A) = -A*kae - A*IL*kl + AIL*k2

d/dt(IL) = kpi - IL*kie - A*IL*kl + AIL*k2

d/dt(AIL) = -AIL*kaie + A*IL*kl - AIL*k2

where kae is the rate of free antibody clearance from the vitreous, k1 is the association rate for antibody/IL-6 binding, k2 is the dissociation rate for the antibody/IL6 complex, kpi is the rate of IL-6 production, kei is the rate of free IL-6 clearance from the vitreous, and kaie is the rate of antibody/IL-6 complex clearance from the vitreous. Starting parameter values and rates were defined as shown in Table 6.

Table 6: Starting parameter values and rates

Parameter	Value
Initial antibody concentration - A_0	3000 nM
Initial IL-6 concentration – IL_0	0.01 nM
Initial complex concentration - AIL_0	0
Association rate – k1	8.64 $nM^{-1}d^{-1}$
Dissociation rate – k2	Varied from 0.0086 d^{-1} to 0.86 d^{-1}
Antibody clearance rate – kae	0.037 d^{-1}
IL6 clearance rate – kie	0.69 d^{-1}
IL6 production rate – kpi	0.0069 nM d^{-1}
Complex clearance rate – kaie	0.037 d^{-1}

A_0 was calculated based on the assumptions of a 50 μL dose of 50 mg/mL antibody into a human eye with a 5 mL vitreal volume. IL_0 was estimated based on clinically measured values for vitreal IL-6 in DME patients of ~200 pg/mL. k1 was estimated based on typical antibody association rates of 1E5 $M^{-}V^{1}$, while k2 was varied to simulate potency values ranging from 100 pM to 1 pM. kae was derived from measured vitreal clearance half-times in the rabbit of ~11 days scaled by 1.8 as previously measured for human PK. kie was estimated at a clearance half time of 24 hours, and kpi was calculated as ILo*kie.

Simulations of free antibody and free IL-6 were performed using Berkeley Madonna software over a 300 day time course (Fig. 10). A cut-off of 95% IL-6 blockade was selected to measure duration of inhibition. The model predicts that increasing the antibody potency significantly extends the duration of IL-6 inhibition in the eye from 130 days for k2/kl = 100 pM to 200 days for k2/kl = 10 pM to 225 days for k2/kl = 1 pM.

Example 21: Pharmacokinetics of IL-6 Antibodies

Pharmacokinetic (PK) experiments were performed in male New Zealand White Rabbits by PharmOptima (Portage, MI). All animals were 12-13 months of age and weighed 2.61 - 3.42 kg. The following proteins were compared - EBI-029-IgG2 (SEQ ID NO:l 1 and SEQ ID

NO: 12), EBI-029-IgG2-H31 1A (SEQ ID NO:10 and SEQ ID NO: 12), EBI-030 (SEQ ID NO:41 and SEQ ID NO:42), EBI-030-IgG2-H31 1A (SEQ ID NO:47 and SEQ ID NO:42), EBI-029 Fab (SEQ ID NO:24 and SEQ ID NO: 12), Eylea® (VEGF trap), and Tocilizumab (TCZ; anti-IL6R antibody). All proteins were formulated at 13.8 mg/mL in PBS, pH 7.4. EBI-029-IgG2, EBI-029-IgG2-H31 1A, EBI-030, EBI-030-IgG2-H31 1A, EBI-029 Fab, and Tocilizumab do not bind to their target antigens in the rabbit, while Eylea® does bind to rabbit VEGF.

For the investigation of intravitreal PK, 9 animals were injected with 50 μL of test article in each eye. Prior to injection, Lidocaine hydrochloride (injectable 2%), 0.5% Proparacaine, or 0.5% Tetracaine was applied to the ocular surface. Injections were performed into the mid-vitreous with a BD 300 μL insulin syringe (31G x 5/16 inch needle) inserted through the dorsotemporal quadrant of the eye. For the investigation of systemic PK, 3 animals were injected with 100 μL of test article through the ear vein.

Serial blood samples were collected from 3 animals in both the IVT and iv arms at 0.083, 1, 4, 8, 24, 72, 168, 240, and 336 hours and diluted 1:1 with Citrate-Phosphate-Dextrose solution and placed on ice. Plasma was harvested by centrifugation of the chilled blood samples at 4000 rpm for 10 minutes at 4°C and stored frozen at -80°C.

Ocular tissues were harvested from both eyes of all animals in the IVT arm at 0.25, 24, 168, and 336 hours post dose. Animals were euthanized via intravenous barbiturate overdose. To harvest aqueous humor, immediately following euthanasia, a syringe with needle was inserted under the cornea and the aqueous humor slowly withdrawn. Aqueous humor was transferred to a pre-labeled tube and placed on dry ice or frozen at -80°C. To harvest vitreous humor, a small slice was introduced in the sclera of an enucleated eye using a scalpel and vireous was withdrawn through the opening via syringe. The sample was measured via the graduations on the syringe, transferred into a pre-labeled tube, and placed on dry ice or frozen at -80°C.

To harvest retina and choroid, a small slice was introduced with a scalpel in the sclera of an enucleated eye, parallel and caudal to the limbus. Scissors were used to continue the opening around the globe of the eye, separating it into two halves. The posterior globe was positioned so that the interior was facing upward. Using a gill knife, retina was carefully collected from the globe. Once retina was collected from the globe, choroid was collected in a similar manner from the remaining globe. Both samples, separately, were transferred to pre-weighed and pre-labeled

Precellys® tubes, weighed, and placed on dry ice or frozen at -80°C. Retina and choroid tissues were diluted ten-fold in Phosphate Buffered Saline (PBS), homogenized, and stored at -80°C.

Protein concentrations in each tissue were assessed by ELISA. For EBI-029-IgG2, EBI-029-IgG2-H31 1A, EBI-030, EBI-030-IgG2-H31 1A, and EBI-029 Fab, Costar half-volume plates were coated with 1 µg/mL human IL-6 in PBS for 1 hour at RT. Wells were blocked with PBS containing 2% BSA, washed, and then incubated with a range of dilutions for each sample using PBS + 5% rabbit plasma + 0.05% Tween-20 as the diluent. A standard curve using purified protein was also included on each plate. Samples were incubated at RT for 60 minutes then washed three times with 300 µï PBS containing 0.05% Tween-20. Anti-kappa-HRP (Genway Inc.) diluted 1:10,000 in PBS, 1% BSA, 0.05% Tween-20 was then added to each well and incubated for 30 minutes. Wells were washed as above then 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added and the signal measured at 450 and 550 nm on a Spectramax plate reader. Protein concentrations were calculated based on the standard curve using Softmax Pro 6 software. Each ELISA was repeated on at least 3 independent plates and the average half-time was reported.

For tocilizumab, protein concentrations were determined by ELISA as above except that anti-Tocilizumab Fab (BioRad HCA252) was used as the capture reagent and anti-human-IgG-Fc-HRP (Sigma A O170) was used as the detection antibody. Two different ELISA assays were used to measure free and total Eylea®. For free Eylea®, wells were coated with recombinant VEGF (**R&D** Systems) and bound protein was detected with anti-human-IgG-Fc-HRP (Sigma A O170). For measuring total Eylea®, anti-human Fc antibody (Sigma 12136) was used for capture and anti-human IgG-CH2-HRP (BioRad MCA647P) was used for detection. Each ELISA was repeated on at least 3 independent plates and the average half-time was reported.

Summary of results

In most animals, robust antibody formation against the injected protein was observed at the 240 and 336 hour timepoints. Because this antibody formation may affect protein clearance or interfere with the ELISA, data analysis was limited to the time points up to and including 168 hours. For intravitreal PK, all of the EBI-029 and EBI-030 IgG2 proteins were cleared significantly more slowly ($Ti/_2$ = 9.3 days, 9.0 days, 15.7 days, and 9.8 days for EBI-029, EBI-029-H31 1A, EBI-030, and EBI-030-H31 1A, respectively) compared to Eylea® ($Ti_{/2}$ = 6.3 days),

Tocilizumab ($Ti/_2$ = 4.8 days), or the EBI-029 Fab fragment ($Ti/_2$ = 3.9 days) (Fig. 11, Table 7). Similar trends were observed in the retina, choroid, and aqueous where EBI-030 and EBI-030-H31 1A accumulated at higher levels compared to Eylea® and Tocilizumab (see Fig. 12 and Fig. 13). All proteins were detectable in the plasma following IVT administration with EBI-029, EBI-030, and Tocilizumab accumulating at significantly higher levels than Eylea® or EBI-030-H3 11A (see Fig. 14). Similarly, Eylea® and EBI-030-H3 11A were cleared more quickly from the plasma following IV administration, with the EBI-030-H31 1A half-time approximately half that of the wild-type IgG2 due to reduced FcRn binding (Table 7).

Table 7: Pharmacokinetic results

Vitreous PK	
Molecule	$T_{1/2}$ (days)
EBI-029	9.3
EBI-029-H311A	9.0
EBI-030	15.7
EBI-030-H311A	9.8
EBI-029 Fab	3.9
Eylea®	6.1 (free), 6.3 (total)
Tocilizumab	4.8

Systemic PK after IV administration	
Molecule	$T_{1/2\beta}$ (hours)
EBI-029	77
EBI-030	69
EBI-030-H311A	33
Eylea®	37 (free), 42 (total)
TCZ	50

Example 22: Determination of Effective Antibody or Effective Fragment

In results described herein, e.g., in Example 21, it was found that EBI-029, EBI-030, and EBI-031 (also referred to as EBI-030-H31 1A) had a surprisingly good ocular retention, e.g., surprisingly long vitreal half life, compared with other agents such as tocilizumab and Eylea®. To determine which antibody sequences or fragments are effective to provide this improved vitreal retention, full length IgG2, Fab, scFv, and Fc versions of an experimental antibody (EBI-

029, EBI-030, and/or EBI-031) are produced. Full length, Fab, scFv, and Fc versions of one or more control antibodies (e.g., tocilizumab and/or bevacizumab) are also produced. These experimental and control antibody sequences and fragments are administered to rabbits as described in Example 21 and pharmacokinetic parameters, such as vitreal half life, are assessed, e.g., as described in Example 21. The results will reveal which portion(s) of the experimental antibody is effective to improve vitreal retention. Smaller portions of any effective antibody or effective fragment can in turn be investigated to determine whether smaller fragments are effective to improve ocular retention.

Example 23: Reducing or ablating IL-6 binding of an effective antibody or effective fragment

If the effective antibody or effective fragment includes antigen-binding portions of the antibody, the effective antibody or effective fragment can be mutated to decrease or ablate IL-6 binding.

In one exemplary method, CDR residues in the effective antibody or effective fragment are mutated singly (e.g., to Ala or Arg residues) and the binding of the resulting mutants is tested (e.g., using SPR) to determine which single mutations disrupt binding. If needed, multiple mutations are combined to generate an effective antibody or effective fragment that shows decreased binding.

In another example, yeast display methods are employed. See, e.g., Chao et al. Nature Protocols vol. 1 no. 2 pp. 755-768, 2006. Mutations (e.g., 1-3 random or targeted mutations per antibody) are introduced and a large number of mutant versions (e.g., 1E7 - 1E9 mutant versions) of the effective antibody or effective fragment are displayed in a yeast display library. Antibody display and binding to IL-6 is tested and mutants that show reduced binding are selected by FACS. Selected clones are sequenced to identify the inactivating mutations.

The selected mutant version(s) of the effective antibody or effective fragment is tested in pharmacokinetic studies to verify that it retains the pharmacokinetic properties of the parent effective antibody or effective fragment. The resulting mutant effective antibody or effective fragment can be covalently attached (e.g., as a fusion protein) to another agent, e.g., using methods described herein and/or methods known in the art.

Example 24: Production of Fusion Proteins

The effective antibody or effective fragment, e.g., as identified in Example 22, is fused to another agent comprising a polypeptide (e.g., a polypeptide comprising therapeutic agent) using standard biotechnology techniques. For instance, PCR is used to amplify DNA encoding the effective antibody or effective fragment and the fusion partner. Primers can be selected to introduce compatible restriction sites at the respective N- and C-termini such that the two PCR fragments can be ligated into an expression vector as an inline fusion. Alternatively, primers can be selected to introduce 10-20 conserved nucleotides at the respective N- and C-termini such that the two PCR products can be combined by overlap extension PCR. The combined PCR product can then be ligated into an expression vector as described above. In some instances, primers can be designed to introduce nucleotides encoding a linker (eg. GS or $(G_4S)_X$) between the two protein domains.

The pharmacokinetics of the resulting fusion protein are investigated using methods described herein and/or known in the art. For example, the methods described in Example 21 can be used to investigate retention of the fusion protein in the eye. It is anticipated that the fusion protein will have better retention in the eye compared with the agent when it is not fused to the effective antibody or effective fragment.

Example 25: EBI-031 solubility at high concentrations

Purified EBI-031 was concentrated from 3 mg/mL to 142 mg/mL in PBS, pH 7.4 using an Amicon Ultra-15 spin concentrator. The pre- and post-concentration preps were assessed for aggregation by running on a Tosoh G3000SWXL 7.8x30 SEC column combined with a Wyatt miniDawn TREOS light scattering instrument and Wyatt Optilab rEX refractive index instrument. 20 µg of protein was injected and run at a flow rate of 1 mL/min in PBS. The mass fraction for the peak at the expected molecular weight of ~150 kDa was approximately equal for the two concentrations (90.9% for the 3 mg/mL and 91.3% for the 142 mg/mL prep) indicating that there was no significant increase in protein aggregation during concentration. These results demonstrate that EBI-031 can be concentrated to up to 142 mg/mL with little measurable aggregation (<10%> aggregation).

Example 26: EBI-031 blocks cis- and trans- IL6 signaling

The HEK-Blue™ IL6 reporter cell line (Invivogen) was used to compare the potency of EBI-03 1 and tocilizumab for blocking cis- and trans- IL6 signaling. For cis- signaling, free IL-6 (final concentration = 20 pM) was mixed with EBI-03 1 or tocilizumab at a range of concentrations in a 96 well plate and incubated at RT for 30 minutes. HEK-Blue™ IL6 cells in log phase were trypsinized and resuspended in assay media (DMEM, 4.5 g/1 glucose, 10% Heat inactivated FBS, 2 mM L-glutamine, Pen-Step), and 50,000 cells were added to each well in a final volume of 200 μL. Plates were incubated at 37°C/5%> $C0_2$ for 20 hours. 50 μL of supernatant from each well was then mixed with 150 μL of Quanti-Blue™ reagent (Invivogen) and incubated at 37°C for 40 minutes before measuring absorbance at 650 nM on a SpectraMax M5 plate reader. The background signal from wells with no IL-6 was subtracted and then divided by IL-6 treated cells with no inhibitor to derive a fractional signaling value. EBI-03 1 (IC50 = 14.2 pM) blocks free IL-6 with >900 fold greater potency compared to tocilizumab (IC50 = 12.9 nM) (Fig. 16A).

To measure trans- signaling blockade, experiments were performed as above except using hyper IL-6 at a final concentration of 200 pM instead of free IL-6. Hyper IL-6 is a genetic fusion between IL-6 and the soluble IL-6 receptor (Fischer et al., Nature Biotechnology 15:142-145 (1997). EBI-031 blocked hyper IL-6 potently (IC50 = 32 pM), while tocilizumab was unable to significantly inhibit signaling out to a 1 μM concentration (Fig. 16B).

These results show that EBI-03 1 binds human IL-6 at site II, or the site that contacts gpl30, with pM affinity and blocks signaling of IL-6 and the IL-6/sIL-6Ra complex in cellular assays >900 fold more potently than tocilizumab.

Example 27: Computational simulations for intravitreal EBI-031 suppression of IL-6 signaling

Computational simulations were performed as described in Example 20 to predict the length of time that an intravitreal administration of EBI-031 in humans should suppress 95% of IL-6 signaling. k2 was set to 0.12 d-1 such that k2/kl = 14 pM as measured in the potency assay. Tl/2 clearance was set to 18 days based on the measured intravitreal clearance half-time in rabbits scaled by 1.8 for humans. All other parameters are described in Table 6. The model predicts that EBI-031 should block 95% of IL-6 signaling for ~150 days after intravitreal

administration (Fig. 17). These modeling results indicate that EBI-031 can substantially block IL-6 signaling in the eye for a long period of time, e.g., up to about 6 months.

Example 28: Pharmacokinetics in primate studies

The pharmacokinetics of EBI-03 1 was investigated in primate studies. Two male African green monkeys were tested. 50µ1of 50 mg/mL of EBI-031 was intravitreally injected into the eye. Madonna software was used for curve fitting.

The data from the primate study was modeled using a curve fit. Differential equations describing the changes in antibody in the vitreous (A) and antibody outside of the vitreous, e.g., systemic, (Ap) were defined as follows:

d/dt(A) = -A*kae

d/dt(Ap) = A*kae(Dil) - Ap*kape

The starting parameter values and rates are defined as shown in the table below:

Table 8: Starting parameter values and rates

Parameter	Value
Dil - Dilution	100
kae – Rate of vitreal elimination	0.2
kape – Rate of systemic elimination	1.4
Init A – Initial Antibody in vitreous	1000000
Init Ap – Initial Antibody outside of vitreous	0

Other considerations included for fit include: dilution and both rate constant were floated for fit. Initial A was held constant (2x50ml of 50 mg/mL in 5 mL eye). The results of the modelling (Fig. 18A, 18B, and 19) showed that vitreal elimination rate constants results in half lives of 4.6 and 5.7 days, respectively for the two monkeys. The average vitreal elimination rate constant was calculated to be 5.2 days. Systemic elimination was modeled as 1.1 days, and 0.63 days (average 0.85 days). These results demonstrate that the half-life of EBI-031 in the vitreous was significantly longer than the systemic half-life in primates.

Example 29: Pharmacokinetics of EBI-031

Another pharmacokinetic (PK) experiment was performed, where 50 µï of a 20 mg/mL solution of EBI-031 was injected intravitreally into the eyes of rabbits. Time points examined were 1, 3, 7 and 14 days (e.g., 24, 72, 168, and 336 hours). Two animals (four eyes) were

analyzed per time point. The methods for administering the EBI-03 1 formulation, harvesting the ocular tissue, and determining protein concentration were performed as described in Example 21.

The results are shown in Figures 20A-20I. When analyzing the protein concentration for days 1-14 in the vitreous humor, the EBI-03 1 half-life was determined to be 8.95 days (Fig. 20A). However, a strong antibody response was detected on Day 14, which can affect these results. When the protein concentration for days 1-7 in the vitreous humor was analyzed, EBI-03 1 half-life was determined to be 18.88 days.

EBI-03 1 was also detected in other compartments of the eye after intravitreal injection. EBI-03 1 had also permeated to the aqueous humor (Fig. 20B), the choroid (Fig. 20C), the conjunctiva (Fig. 20D), the cornea (Fig. 20E), the ciliary body (Fig. 20F), the lens (Fig. 20G), the retina (Fig. 20H), and the sclera (Fig. 201). The drug concentration in these tissues were one to two orders of magnitude lower than the concentrations detected in the vitreous.

Example 30: Comparison of the pharmacokinetics of IgGl or IgG2 anti-IL-6 antibodies

The contribution of the IgG2 constant region on the ocular retention, e.g., half-life, of anti-IL-6 antibodies was tested. Two anti-IL-6 antibodies were used for this experiment, EBI-030 which contains an IgG2 constant region, and tocilizumab, which contains an IgGl constant region. The sequence for EBI-030 is provided in Table 4, and differs from EBI-03 1 by one amino acid, H31 1A. An IgGl variant of EBI-030 was engineered to replace the IgGl portion of the heavy chain sequence with an IgG2 sequence. The resulting engineered EBI-030 IgGl heavy chain sequence is SEQ ID NO: 39 in Table 4.

Tocilizumab is an anti-IL-6 antibody with an IgGl constant region. Tocilizumab has heavy and light chain sequences as follows:

Heavy chain IgGl sequence (IgGl portion underlined):

QVQLQESGPGLVRPSQTLSLTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYISYSGITTY
NPSLKSRVTMLRDTSKNQFSLRLSSVTAADTAVYYCARSLARTTAMDYWGQGSLVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLY SL SSVVTVP SSSLGTQT YICN VNHKP SNTKVDKKVEPKSCDKTHTCPP CPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS

REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWOOGNVFSCSVMHEALHNHYTOKSLSLSPGK
(SEQ ID NO:69).

Light chain sequence:
DIQMTQSPSSLSASVGDRVTITCRASQDISSYLNWYQQKPGKAPKLLIYYTSRLHSGVPS
RFSGSGSGTDFTFTISSLQPEDIATYYCQQGNTLPYTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO:70).

Tocilizumab is engineered to replace the IgGl portion of the heavy chain sequence with an IgG2 sequence. The resulting engineered tocilizumab heavy chain sequence is as follows:
QVQLQESGPGLVRPSQTLSLTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYISYSGITTY
NPSLKSRVTMLRDTSKNQFSLRLSSVTAADTAVYYCARSLARTTAMDYWGQGSLVTVS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:71).
A third tocilizumab construct was engineered with mutations to lower the isoelectric point of the molecule (TCZ X3 IgGl), as described in Tsunoda et al, Protein Engineering, Design & Selection, 2010, 23(5):385-392.

The pharmacokinetics of these constructs was assessed in the eyes of rabbits. Formulations of each of the constructs (EBI-030 IgGl, EBI-030 IgG2, TCZ IgGl, TCZ IgG2, and TCZ X3 IgGl) was prepared at 3 mg/ml. The proteins were combined into groups 1 and 2 for testing in rabbits, as shown in Table 9 below. Specifically, Group 1 contained EBI-030 IgGl, TCZ IgG2, and TCZ X3 IgGl . Each protein was present at 3 mg/ml, for a total of 9 mg/ml of total protein formulation injected into rabbits for Group 1. Group 2 contained EBI-030 IgG2 and TCZ IgGl . Each protein was present at 2 mg/ml, for a total of 6 mg/ml of total protein formulation injected into the rabbits for Group 2.

Table 9: Summary of Treatment Groups and PK ELISA

	Capture molecule	Detection	Groups
EBI-030 IgG1	IL-6	α-IgG1	1
TCZ IgG2	α-TCZ FAB	α-IgG2	1
TCZ X3 IgG1	IL-6R	α-IgG1	1
EBI-030 IgG2	IL-6	α-IgG2	2
TCZ IgG1	α-TCZ FAB	α-IgG1	2

Samples were harvested at 20 minutes, 24 hours (1 day), 72 hours (3 days), 168 hours (7 days). The methods for administering the protein formulations, harvesting the ocular tissue, and determining protein concentration were performed as described in Example 21. Detection of protein concentration in the harvested ocular tissue was performed by ELISA assay. Capture molecule that was coated on the bottom of the ELISA plate (e.g., for capture of the anti-IL6 antibody) and the detection reagent for each tested protein is shown in Table 9.

Results of the pharmacokinetic analysis of two trials in the vitreous of the eye is shown in Table 10 below, and in Figure 21A and 21B. In the table below, the protein concentration was determined in μg/ml in the vitreous.

Table 10: Pharmacokinetic analysis of tested proteins in the vitreous

	Time (hours)					
Samples	**0.3**	**24.0**	**72.0**	**168.0**	**Half-life (days)**	**PI**
EBI-030 IgG1	102.5	107.4	68.5	40.6	4.85	6.89
	114.2	110.8	87.6	55.7	6.49	
EBI-030 IgG2	101.6	99.1	75.5	64.3	10.10	6.27
	120.2	134.4	112.1	73.7	8.70	
(total IgG2)	109.55	110.50	89.42	55.17	6.67	
TCZ IgG1	90.0	88.3	65.3	36.8	5.18	8.65

	100.1	102.7	91.2	55.4	7.70	
TCZ IgG2	93.7	96.1	66.0	39.5	5.23	8.39
	95.4	92.3	68.5	42.5	5.75	
TCZ X3 IgG1	88.2	105.7	66.6	46.6	6.42	5.92
	88.3	96.0	67.2	51.9	8.04	

As shown by the data presented here, EBI-030 IgG2 exhibits increased ocular retention in the vitreous with half-life of 8.7 or 10.1 days, as compared to the half-lives of less 5-7 days for the other molecules. However when the constant region of EBI-030 was replaced with IgGl, the extended half-life was reduced to between 4.9-6.5 days. The IgG2 constant region was not sufficient for conferring extended half-life to tocilizumab, as the half-life for TCZ IgG2 was not increased compared to the half-life for the wild-type TCZ (TCZ IgGl). Thus, these data demonstrate that the IgG2 constant region plays a role in ocular retention, but that there are other important sequences that play a role in extending ocular retention in the eye.

Similar pharmacokinetic analysis of the retina was performed (Figure 22 and Table 11) and similar results as those observed in the vitreous was observed.

Table 11: Pharmacokinetic analysis of tested proteins in the retina

	Time (hours)				
Samples	0.25	24	72	168	PI
EBI-030 IgG1	1.89	2.69	2.76	1.59	6.89
EBI-030 IgG2	3.08	3.17	3.45	1.74	6.27
TCZ IgG1	2.82	2.70	2.43	1.47	8.65
TCZ IgG2	1.64	2.28	2.69	1.42	8.39
TCZ X3 IgG1	1.21	1.68	1.84	1.10	5.92

Taken together, these results demonstrate that the IgG2 constant region plays a role in increasing the ocular retention of a molecule, e.g., EBI-030, and that other sequences in EBI-030 also play a role for increasing ocular retention.

Other embodiments are within the scope of the following claims.

What is claimed is:

1. A composition comprising an ocular half life extending (OHLE) polypeptide coupled to an agent, wherein the OHLE comprises

a) a heavy chain module comprising a heavy chain variable region module and an IgG2 constant region module; and

b) a light chain module comprising a light chain variable region module.

2. The composition of claim 1, wherein the heavy chain variable region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with a heavy chain variable region of an antibody in Table 4.

3. The composition of claim 1 or 2, wherein the heavy chain variable region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with SEQ ID NO: 37.

4. The composition of any one of the preceding claims, wherein the IgG2 constant region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with an IgG2 constant region of an antibody in Table 4.

5. The composition of any one of the preceding claims, wherein the IgG2 constant region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with SEQ ID NO: 73 or 74.

6. The composition of any one of the preceding claims, wherein the light chain variable region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with a light chain variable region of an antibody in Table 4.

7. The composition of any one of the preceding claims, wherein the light chain variable region module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity with SEQ ID NO: 38.

8. The composition of any one of the preceding claims, wherein the heavy chain module comprises at least 60, 70, 75, 80, 85, 90, 95, or 99% identity to the framework region of SEQ ID NOs: 41 or 47.

9. The composition of any one of the preceding claims, wherein one or more of the CDRs of the heavy chain module differ from the corresponding heavy chain CDRs of an antibody in Table 4 by 1, 2, 3, 4, 5, 6, 7 or more residues.

10. The composition of any one of the preceding claims, wherein one or more of the CDRs of the light chain module differ from the corresponding light chain CDRs of an antibody in Table 4 by 1, 2, 3, 4, 5, 6, 7 or more residues.

11. The composition of any one of the preceding claims, wherein the composition has increased ocular retention when administered, e.g., intravitreally, to a subject compared with the ocular retention of the agent when it is not covalently attached to the effective antibody or effective fragment thereof.

12. The composition of any of the preceding claims, wherein the ocular retention of the composition in the vitreous, aqueous humor, retina, and/or choroid is increased.

13. The composition of any of the preceding claims, wherein the retention is increased as indicated by an increase in the half life of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%.

14. The composition of any of the preceding claims, wherein the increase in half life is measured in an assay described herein, e.g., an immunosuppression rabbit model.

15. The composition of any of the preceding claims, wherein the half life of the composition in the vitreous of a subject is at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 21, 22, 23, 24, or 25 days when the composition is administered intravitreally to the subject.

16. The composition of claim 15, wherein the subject is a human.

17. The composition of any one of the preceding claims, wherein the OHLE polypeptide binds IL-6, e.g., with an affinity that is at least 50% of that of an antibody from Table 4.

18. The composition of any one of the preceding claims, wherein the OHLE polypeptide does not substantially bind to IL-6, e.g., or binds with an affinity that is less than 20%, 10%, or 5% of that of an antibody from Table 4.

19. The composition of any of the preceding claims, wherein the agent is a therapeutic agent or a diagnostic agent.

20. The composition of any of the preceding claims, wherein the agent is a VEGF antagonist, IL-6 antagonist, IL-1 antagonist, complement inhibitor, or a PDGF pathway antagonist.

21. The composition of claim 20, wherein the VEGF antagonist is aflibercept (Eyelea®), bevacizumab (Avastin®), ranibizumab (Lucentis®), or a fragment thereof that binds VEGF.

22. The composition of claim 20, wherein the IL-6 antagonist is tocilizumab, sarilumab, siltuximab, or a fragment thereof that binds IL-6.

23. The composition of claim 20, wherein the IL-1 antagonist is arcalyst, canakinumab, or a fragment thereof that binds IL-1.

24. The composition of claim 20, wherein the complement inhibitor is lampalizumab (anti-Factor D) or a fragment thereof that binds anti-Factor D.

25. The composition of claim 20, wherein the PDGF pathway antagonist is Fovista® or a fragment thereof that binds PDGF or a PDGFR receptor.

26. The composition of any one of the preceding claims, wherein the agent is coupled to the OHLE via a linker.

27. The composition of claim 26, wherein the linker is a PEG linker.

28. The composition of claim 26, wherein the linker is a peptide linker.

29. The composition of claim 28, wherein the peptide linker comprises one or more of the sequences (G4S)x (SEQ ID NO: 75), (GS)x, (GSS)x, (GGGS)x (SEQ ID NO: 76), (GGGGS)x (SEQ ID NO: 77), (GGSG)x (SEQ ID NO: 78), GSAR (SEQ ID NO: 79), AS, and SS.

30. The composition of any of the preceding claims, wherein the agent is coupled to the N terminus of the heavy chain module.

31. The composition of any of the preceding claims, wherein the agent is coupled to the C terminus of the heavy chain module.

32. The composition of any of the preceding claims, wherein the agent is coupled to the N terminus of the light chain module.

33. The composition of any of the preceding claims, wherein the agent is coupled to the C terminus of the light chain module.

34. The composition of any of the preceding claims, wherein two or more agents are coupled to the OHLE polypeptide.

35. The composition of any of the preceding claims for use in the treatment of a subject having an ocular disease.

36. The composition of any of the preceding claims for use in the preparation of a medicament for the treatment of an ocular disease.

37. The composition of claim 35 or 36, wherein the ocular disease is selected from the group consisting of macular edema (e.g., diabetic macular edema (DME)), uveitis, dry eye (e.g., dry eye disease or dry eye syndrome), allergic conjunctivitis, ocular pain, macular degeneration (e.g., age related macular degeneration (AMD), e.g., wet AMD or dry AMD), retinopathy (e.g., diabetic retinopathy, e.g., proliferative diabetic retinopathy (PDR)), Rhegmatogenous retinal detachment (RRD), retinal vein occlusion (RVO), neuromyelitis optica (NMO), a back of the eye disease, glaucoma, corneal transplant, corneal abrasion, or physical injury to the eye.

38. The composition of any of the preceding claims, wherein the composition is suitable for ocular administration, e.g., intraocular or intravitreal administration.

39. The composition of claim 38, wherein the administration route is intraocular or intravitreal administration.

40. A pharmaceutical composition comprising the composition of any of claims 1 to 39, and a pharmaceutically acceptable carrier.

41. The pharmaceutical composition of claim 40, wherein the pharmaceutical composition is suitable for ocular administration, e.g., intraocular or intravitreal administration.

42. A method of treating an ocular disease comprising administering the composition of any of claims 1 to 39 or a pharmaceutical composition of claim 40 or 41 to a subject in need thereof.

43. A method of claim 42, wherein the composition or pharmaceutical composition is administered by intravitreal administration.

44. The method of claim 43, wherein the ocular disease is selected from the group consisting of macular edema (e.g., diabetic macular edema (DME)), uveitis, dry eye (e.g., dry eye disease or dry eye syndrome), allergic conjunctivitis, ocular pain, macular degeneration (e.g., age related macular degeneration (AMD), e.g., wet AMD or dry AMD), retinopathy (e.g., diabetic retinopathy, e.g., proliferative diabetic retinopathy (PDR)), Rhegmatogenous retinal detachment (RRD), retinal vein occlusion (RVO), neuromyelitis optica (NMO), a back of the eye disease, glaucoma, corneal transplant, corneal abrasion, or physical injury to the eye.

45. The method of claim 43 or 44, wherein the subject is a mammal.

46. The method of any of claims 43 to 45, wherein the subject is a human.

47. A method of making an OHLE polypeptide comprising:

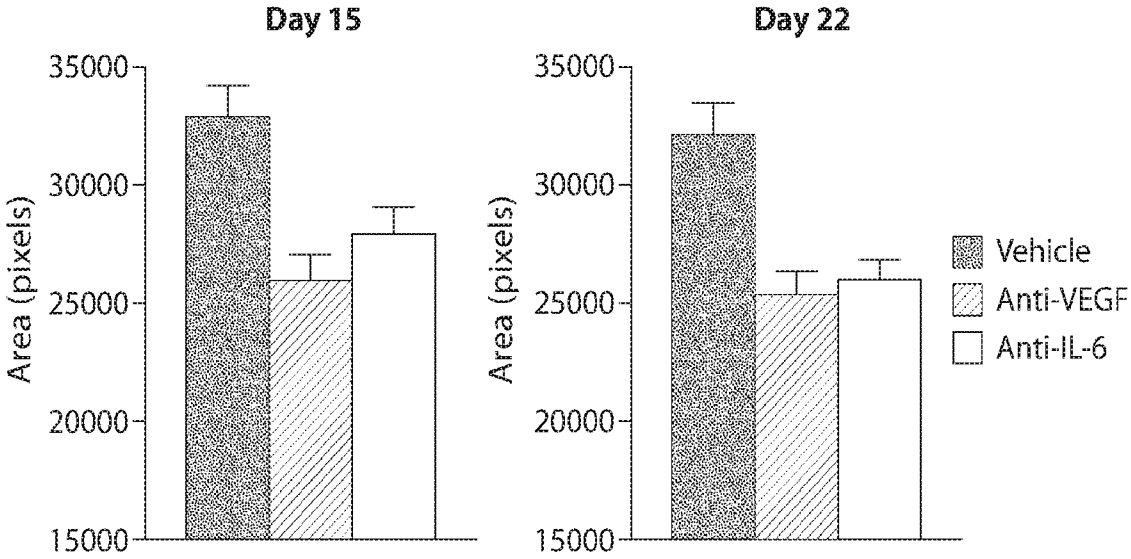
providing an antibody;

altering the sequence of the antibody such that is more similar to the sequence of an antibody in Table 4;

thereby making an OHLE polypeptide.

48. A method of making an improved therapeutic or diagnostic agent comprising coupling, e.g., by making a fusion protein, an OHLE polypeptide to a therapeutic or diagnostic agent.

49. A method of increasing the ocular retention, e.g., half-life, of an agent comprising coupling, e.g., by making a fusion protein, an OHLE polypeptide to a therapeutic or diagnostic agent.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15000
Area (pixels)
Vehicle
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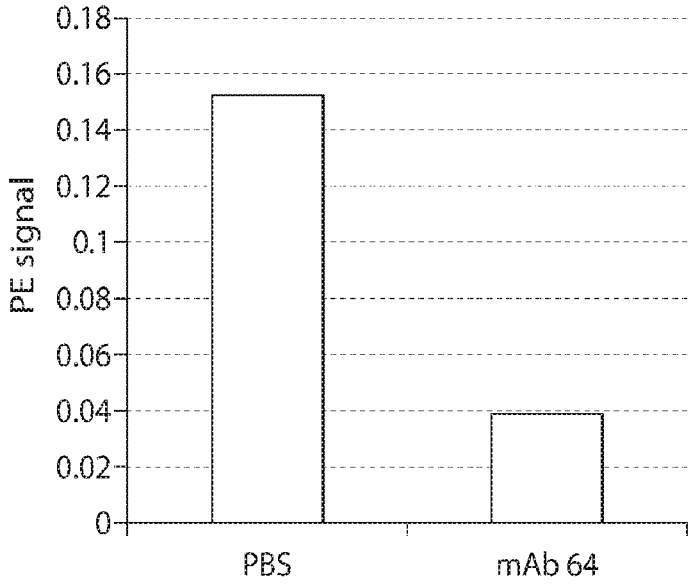
Anti-IL-6

Fig. 1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PBS
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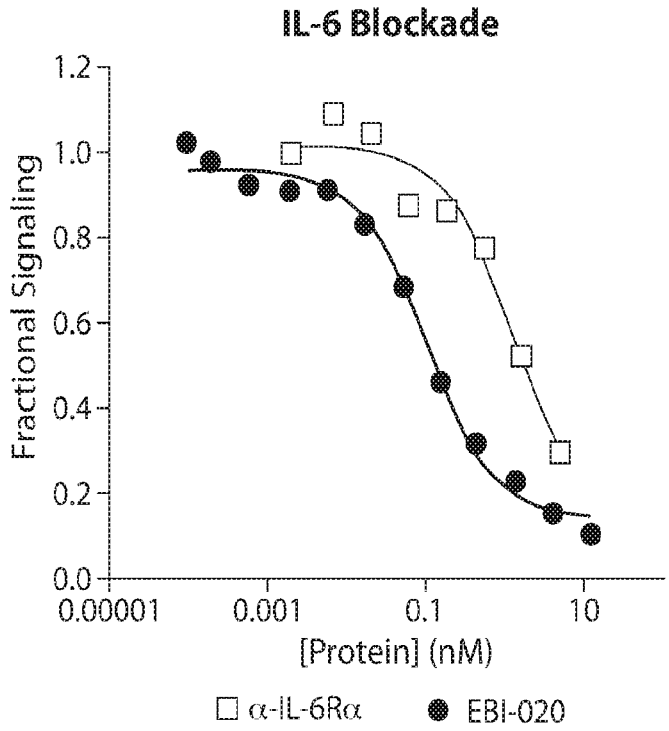
Fig. 2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0.6
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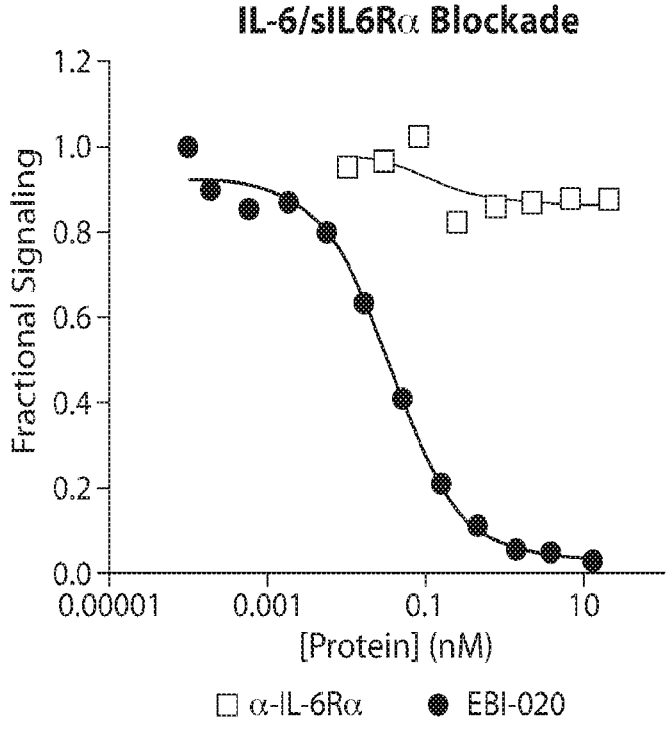
EBI-020

Fig. 3A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Fractional Signaling
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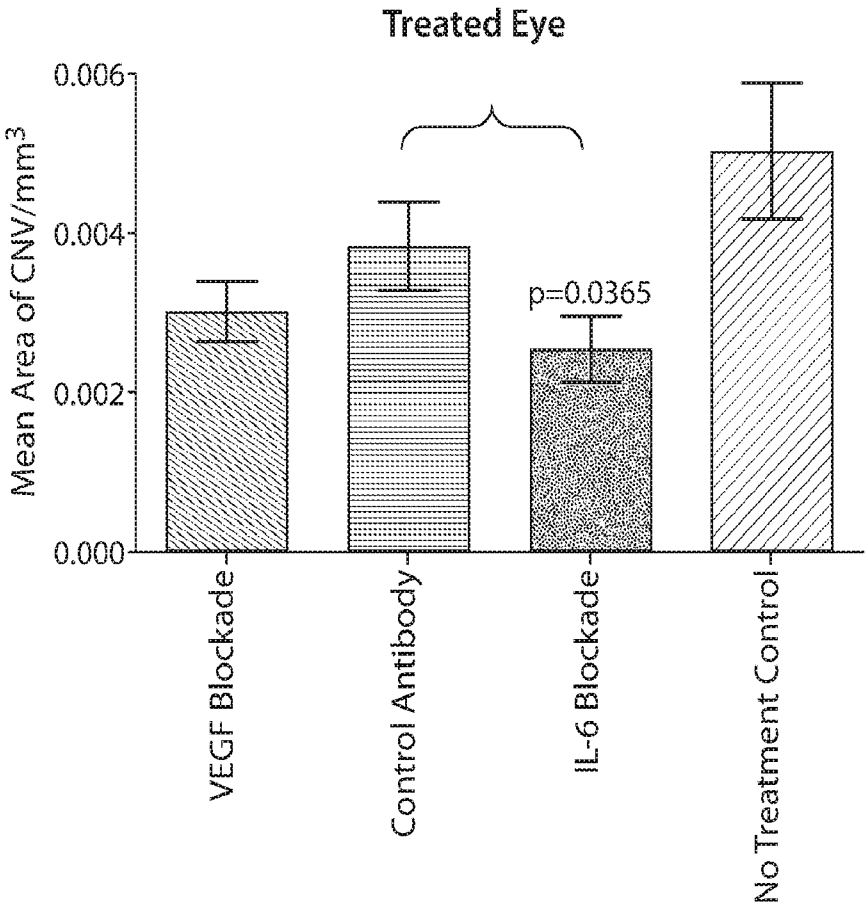
EBI-020

Fig. 3B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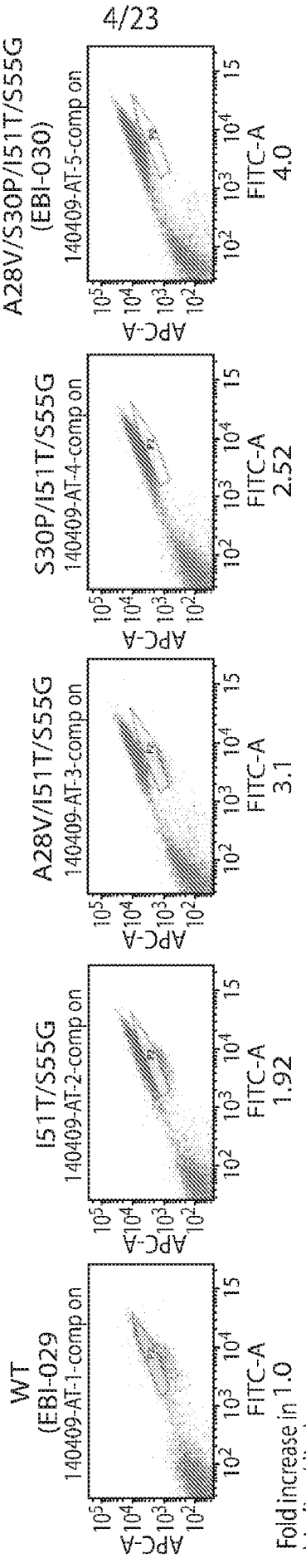
VEGF Blockade
Control Antibody
IL-6 Blockade
No Treatment Control

Fig. 4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FITC-A
Fold increase in 1.0
binding/display
over wt
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A28V/I51T/S55G
140409-AT-3-comp on
3.1
S30P/I51T/S55G
140409-AT-4-comp on
2.52
A28V/S30P/I51T/S55G
(EBI-030)
140409-AT-5-comp on
4.0

Fig. 5

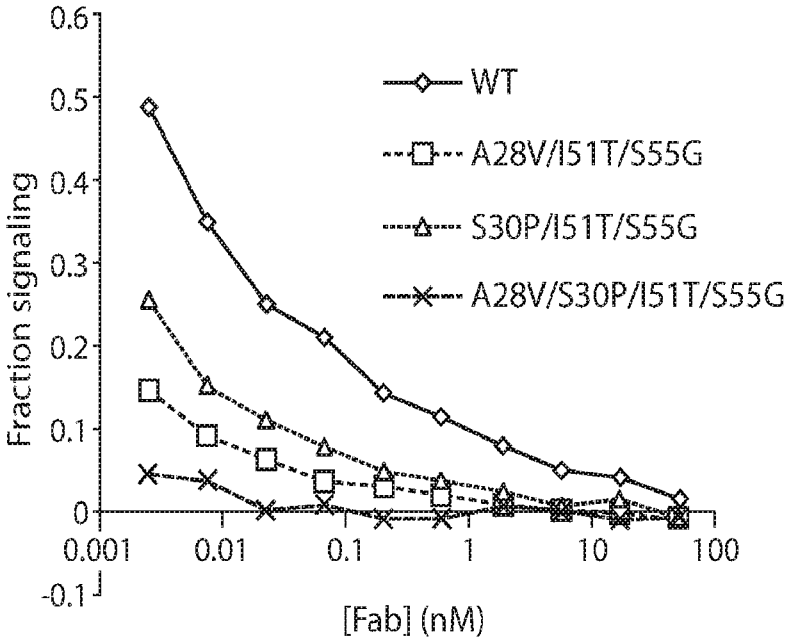
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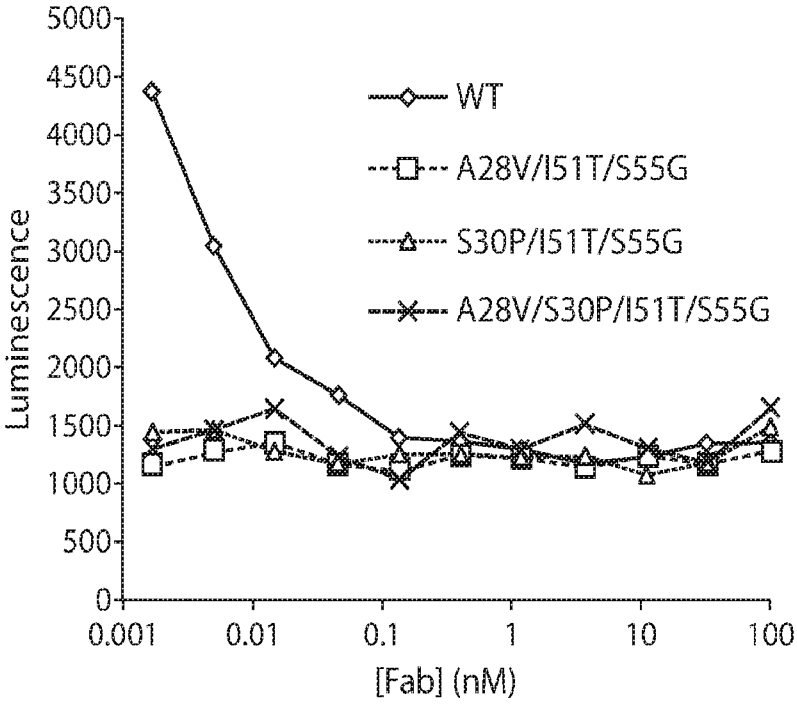
Fig. 6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3000
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2000
1500
1000
500
0
Luminescence
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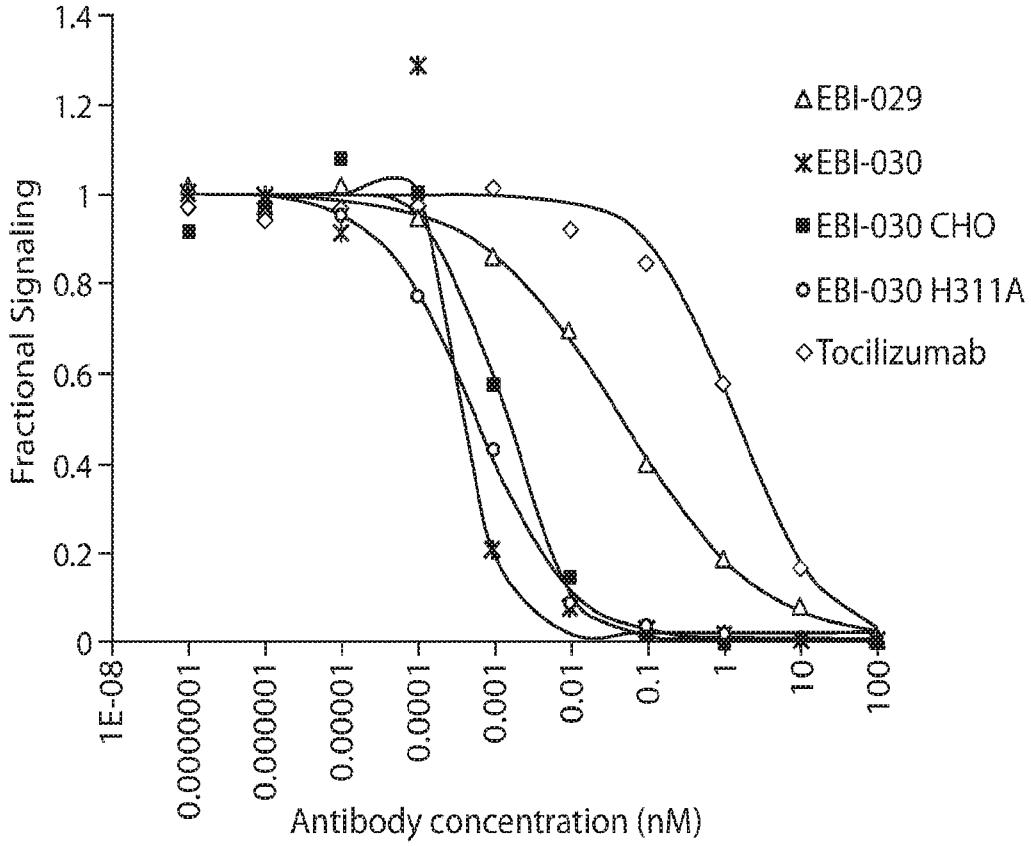
0.01
0.1
1
10
100
[Fab] (nM)
WT
A28V/I51T/S55G
S30P/I51T/S55G
A28V/S30P/I51T/S55G

Fig. 7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0.6
0.4
0.2
0
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0.00001
0.0001
0.001
0.01
0.1
1
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Antibody concentration (nM)
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EBI-030
EBI-030 CHO
EBI-030 H311A
Tocilizumab

Fig. 8

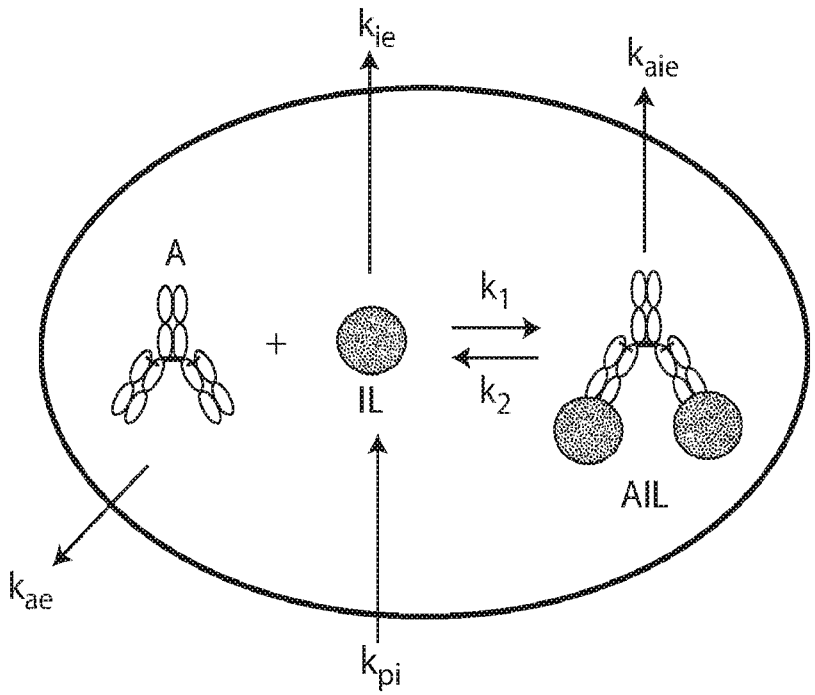
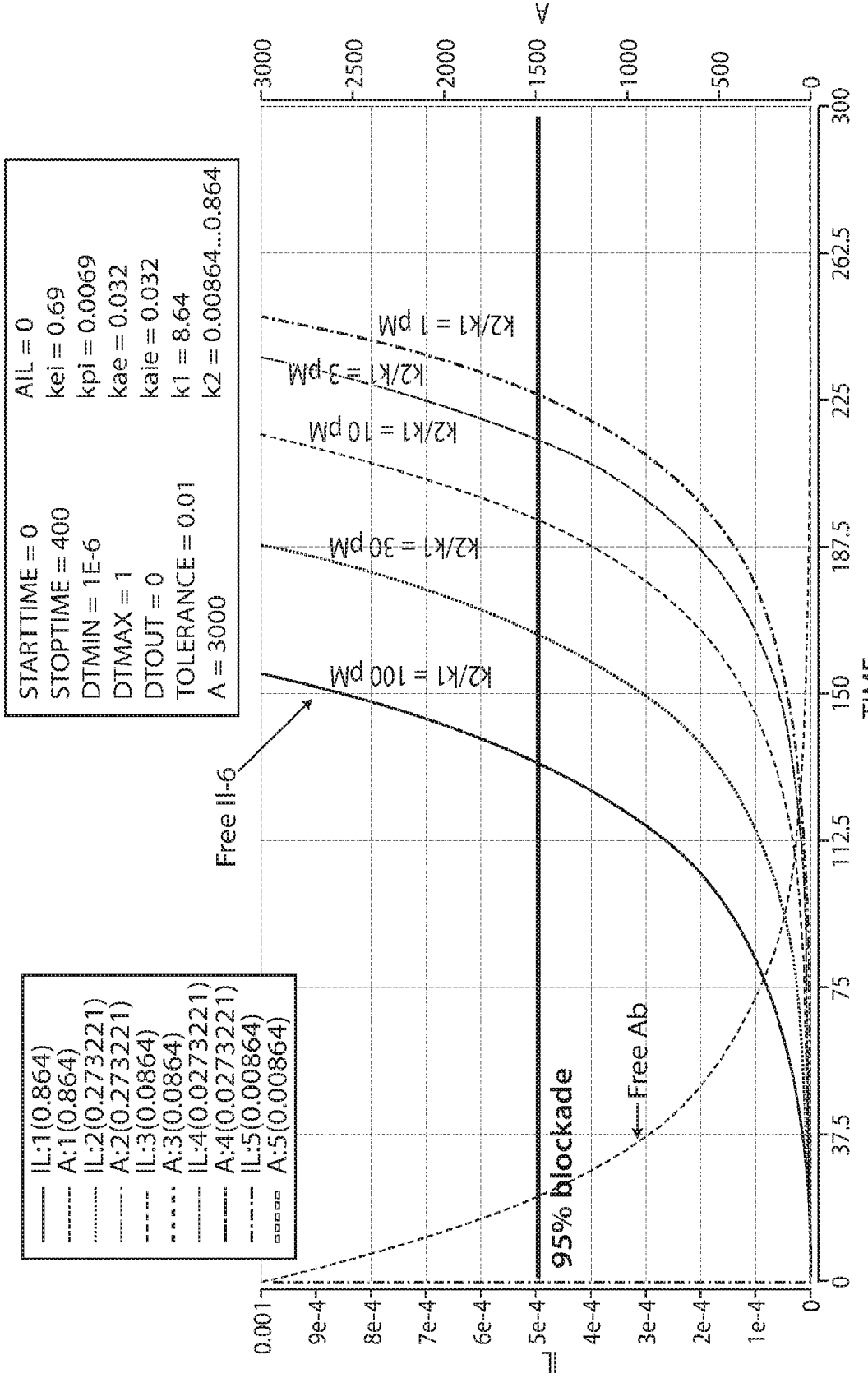


Fig. 9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STOP TIME = 400
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DTOUT = 0
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AIL = 0
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kpi = 0.0069
kae = 0.032
kaie = 0.032
k1 = 8.64
k2 = 0.00864...0.864
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k2/k1 = 100 pM
k2/k1 = 30 pM
k2/k1 = 10 pM
k2/k1 = 3 pM
k2/k1 = 1 pM
95% blockade
Free Ab
A
TIME

Fig. 10

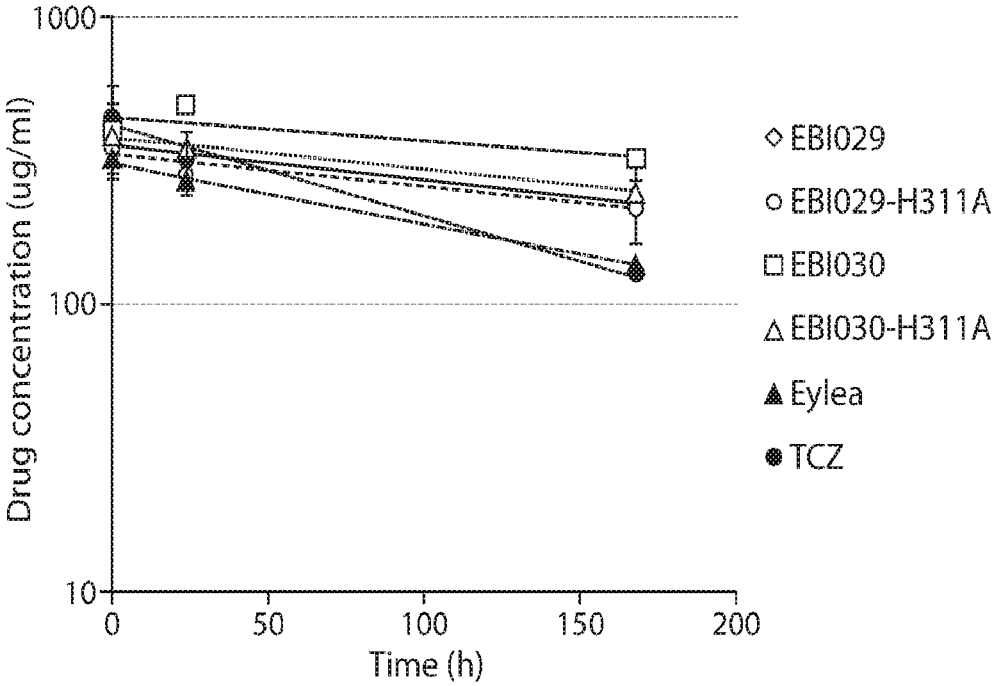
1000
100
10
Drug concentration (ug/ml)
0
50
100
150
200
Time (h)
EBI029
EBI029-H311A
EBI030
EBI030-H311A
Eylea
TCZ

Fig. 11

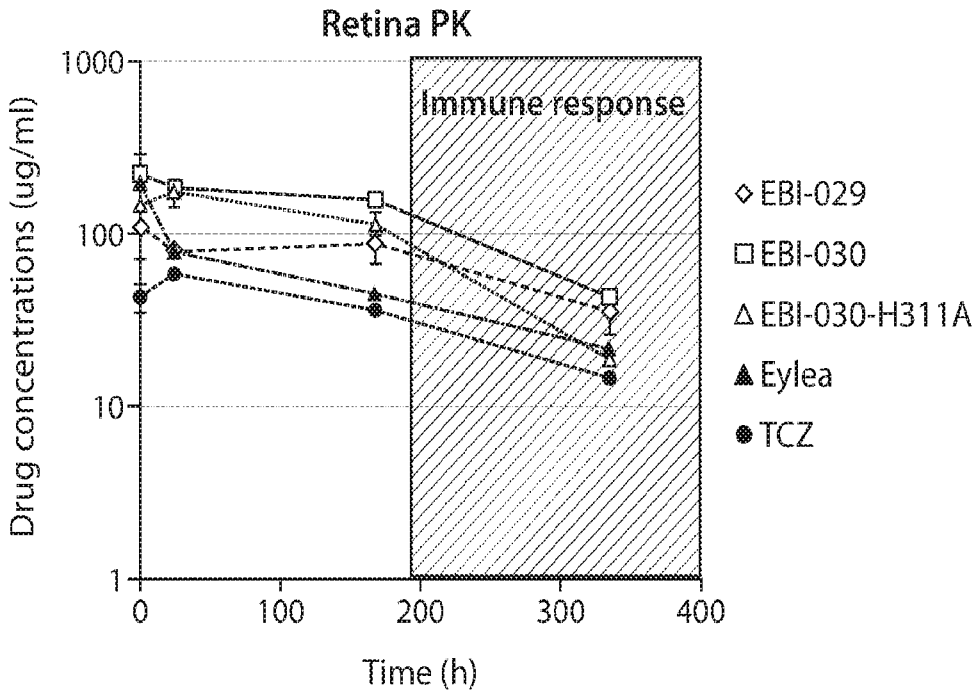
Retina PK
Immune response
Drug concentrations (ug/ml)
1000
100
10
1
0
100
200
300
400
Time (h)
EBI-029
EBI-030
EBI-030-H311A
Eylea
TCZ

Fig. 12

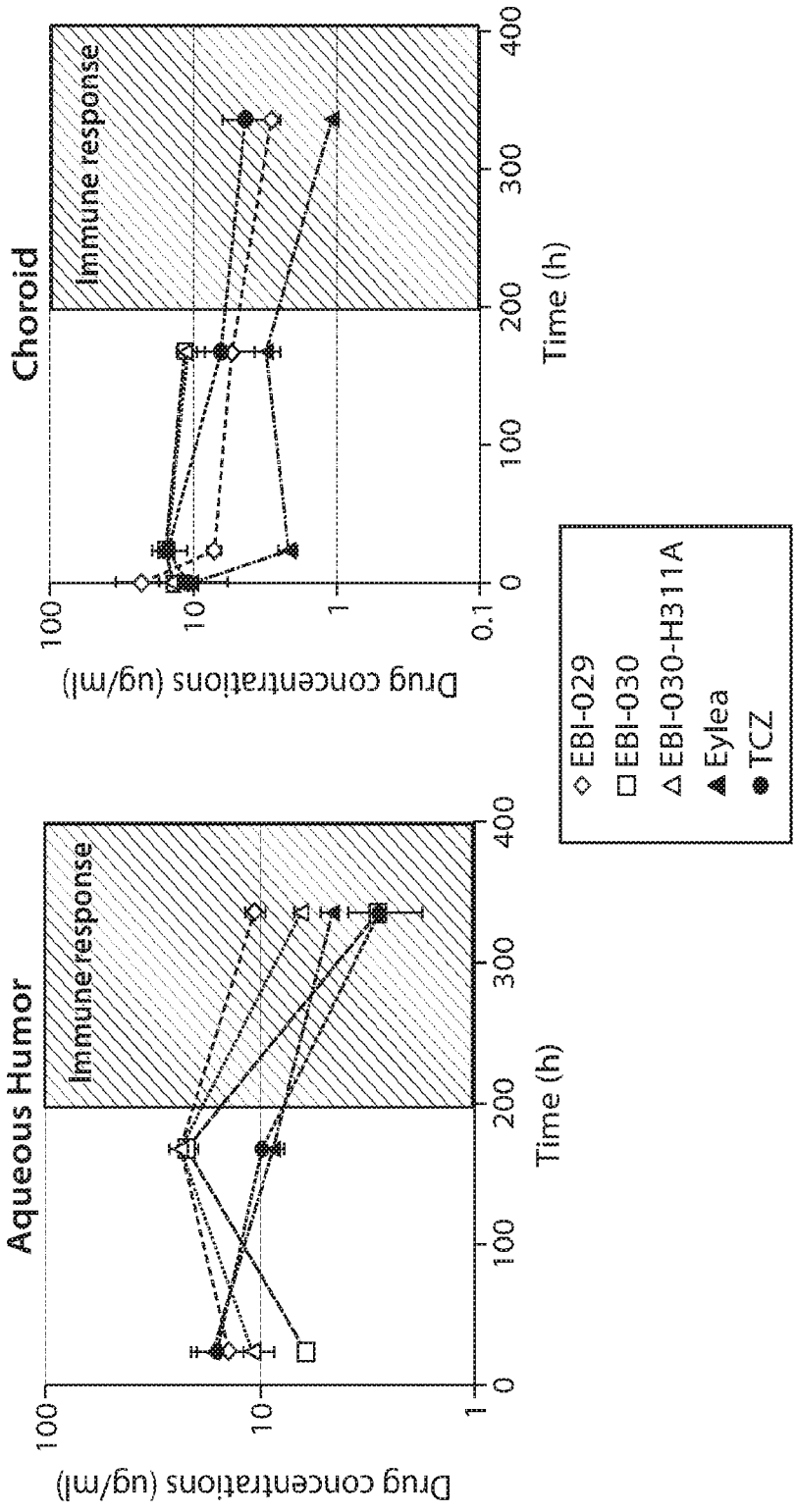
Choroid
Immune response
Drug concentrations (ug/ml)
100
10
1
0.1
0
100
200
300
400
Time (h)
Aqueous Humor
Immune response
Drug concentrations (ug/ml)
100
10
1
0
100
200
300
400
Time (h)
EBI-029
EBI-030
EBI-030-H311A
Eylea
TCZ

Fig. 13

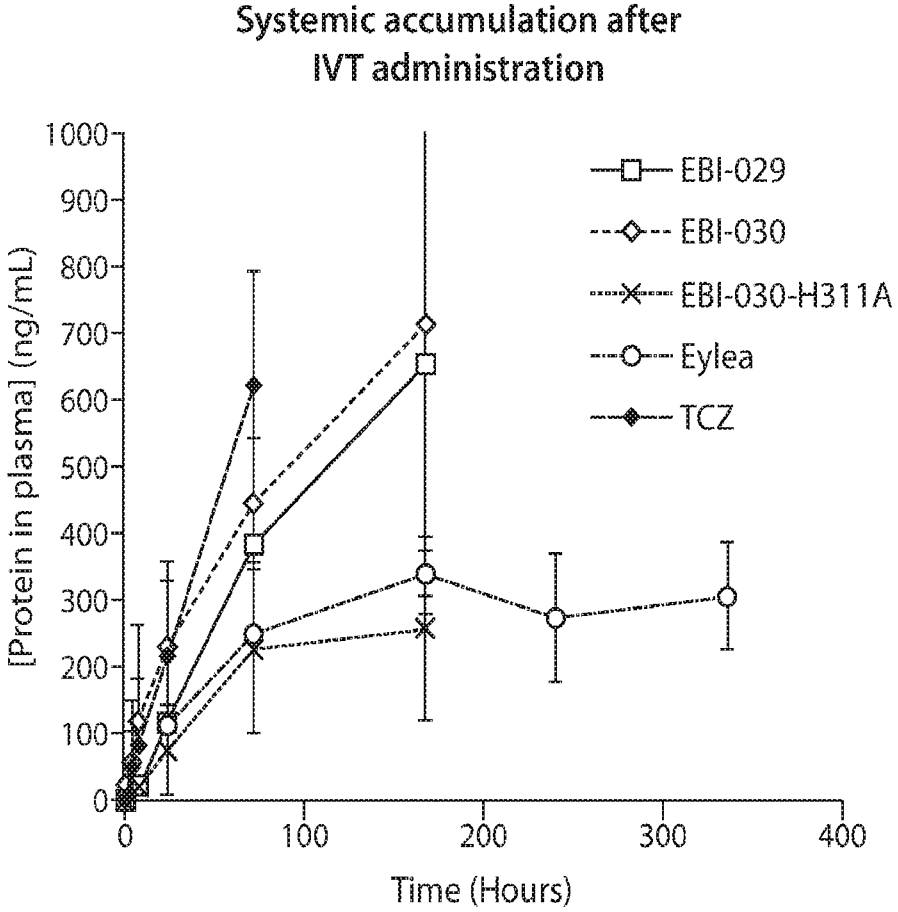
Systemic accumulation after
IVT administration
[Protein in plasma] (ng/mL)
1000
900
800
700
600
500
400
300
200
100
0
0
100
200
300
400
Time (Hours)
EBI-029
EBI-030
EBI-030-H311A
Eylea
TCZ

Fig. 14

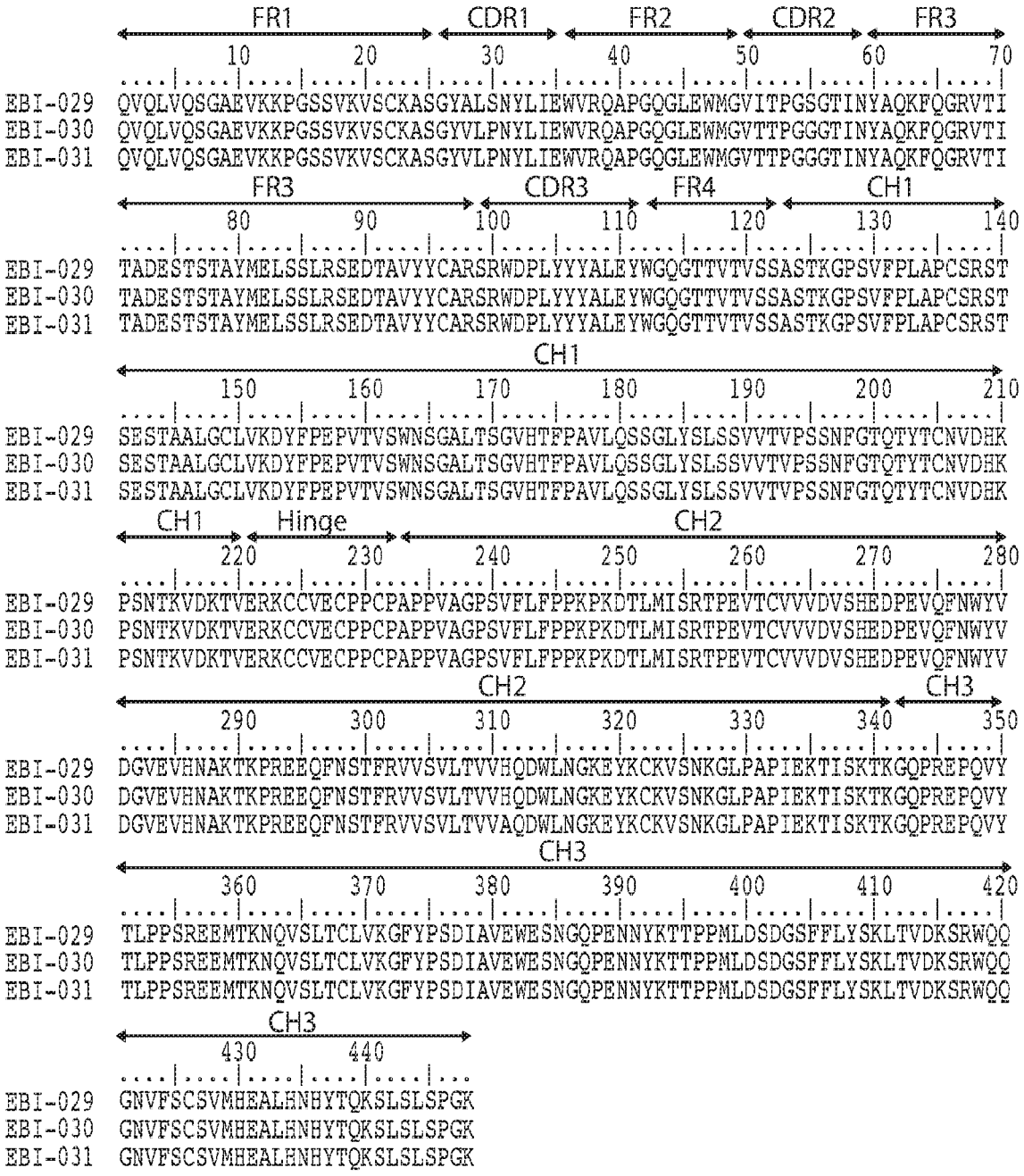
FR1 CDR1 FR2 CDR2 FR3
10 20 30 40 50 60 70
EBI-029 QVQLVQSGAEVKKPGSSVKVSCKASGYALSNYLIEWVRQAPGQGLEWMGVITPGSGTINYAQKFQGRVTI
EBI-030 QVQLVQSGAEVKKPGSSVKVSCKASGYVLPNYLIEWVRQAPGQGLEWMGVTTPGGGTINYAQKFQGRVTI
EBI-031 QVQLVQSGAEVKKPGSSVKVSCKASGYVLPNYLIEWVRQAPGQGLEWMGVTTPGGGTINYAQKFQGRVTI
FR3 CDR3 FR4 CH1
80 90 100 110 120 130 140
EBI-029 TADESTSTAYMELSSLRSEDTAVYYCARSRWDPLYYYALEYWGQGTTVTVSSASTKGPSVFPLAPCSRST
EBI-030 TADESTSTAYMELSSLRSEDTAVYYCARSRWDPLYYYALEYWGQGTTVTVSSASTKGPSVFPLAPCSRST
EBI-031 TADESTSTAYMELSSLRSEDTAVYYCARSRWDPLYYYALEYWGQGTTVTVSSASTKGPSVFPLAPCSRST
CH1
150 160 170 180 190 200 210
EBI-029 SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHK
EBI-030 SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHK
EBI-031 SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHK
CH1 Hinge CH2
220 230 240 250 260 270 280
EBI-029 PSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
EBI-030 PSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
EBI-031 PSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
CH2 CH3
290 300 310 320 330 340 350
EBI-029 DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVY
EBI-030 DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVY
EBI-031 DGVEVHNAKTKPREEQFNSTFRVVSVLTVVAQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVY
CH3
360 370 380 390 400 410 420
EBI-029 TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ
EBI-030 TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ
EBI-031 TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ
CH3
430 440
EBI-029 GNVFSCSVMHEALHNHYTQKSLSLSPGK
EBI-030 GNVFSCSVMHEALHNHYTQKSLSLSPGK
EBI-031 GNVFSCSVMHEALHNHYTQKSLSLSPGK

Fig. 15A

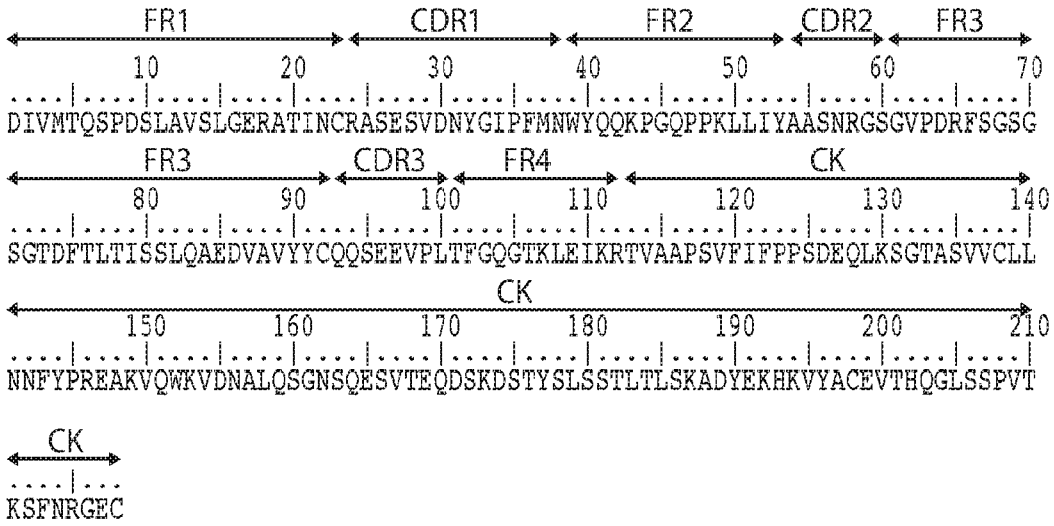
FR1
CDR1
FR2
CDR2
FR3
10 20 30 40 50 60 70
DIVMTQSPDSLAVSLGERATINCRASESVDNYGIPFMNWYQQKPGQPPKLLIYAASNRGSGVPDRFSGSG
FR3
CDR3
FR4
CK
80 90 100 110 120 130 140
SGTDFTLTISSLQAEDVAVYYCQQSEEVPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
CK
150 160 170 180 190 200 210
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT
CK
KSFNRGEC

Fig. 15B

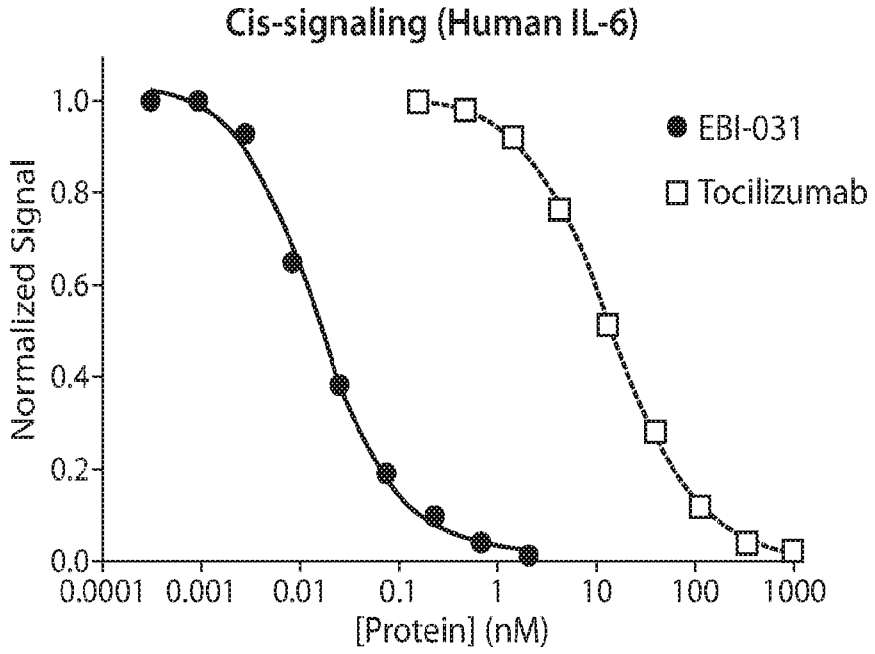

Fig. 16A

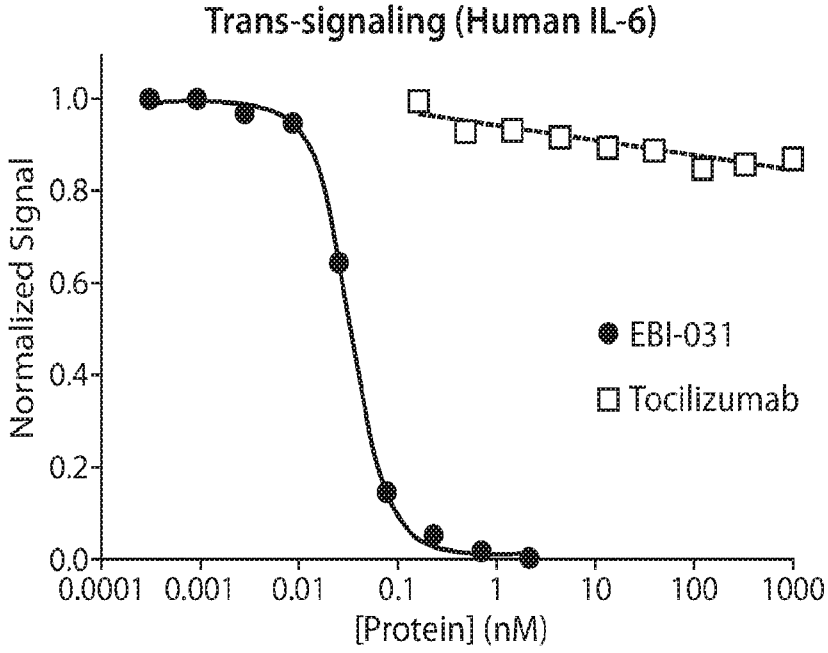

Fig. 16B

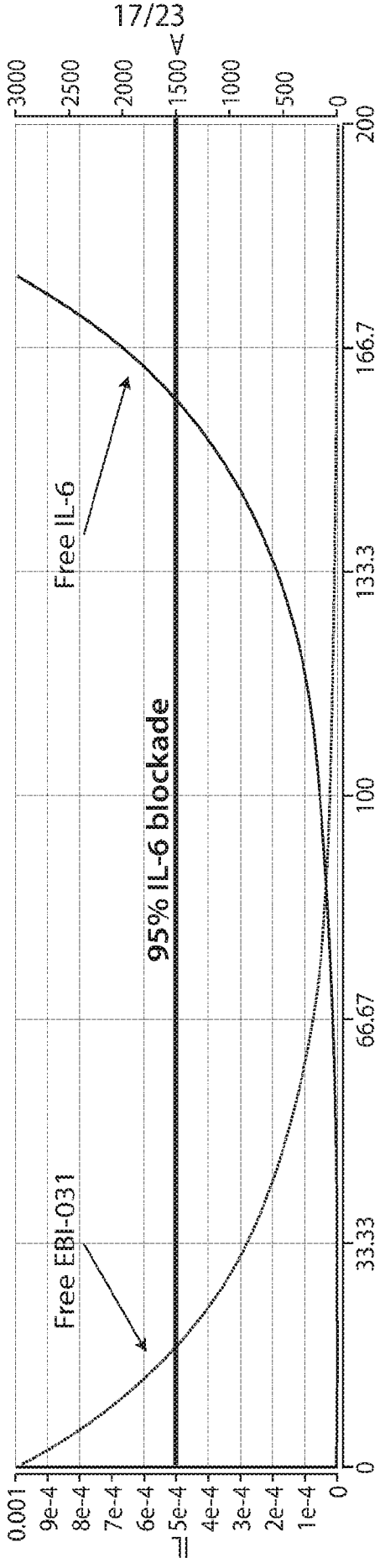
3000
2500
2000
1500
1000
500
0
200
166.7
133.3
100
66.67
33.33
0
TIME
Free IL-6
95% IL-6 blockade
Free EBI-031
0.001
9e-4
8e-4
7e-4
6e-4
5e-4
4e-4
3e-4
2e-4
1e-4
0

Fig. 17

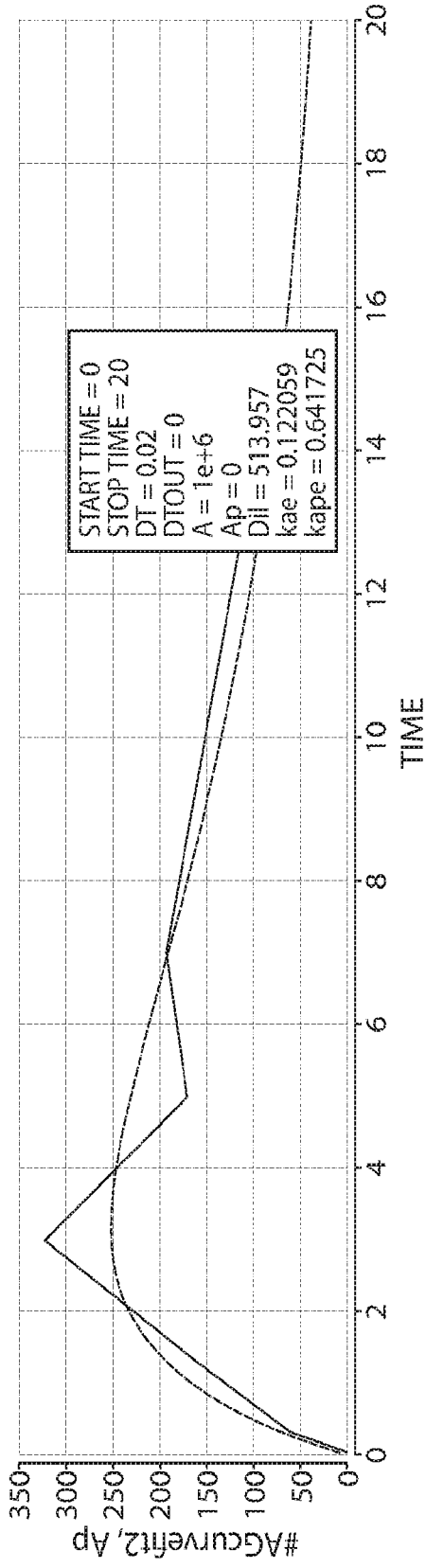

Fig. 18A

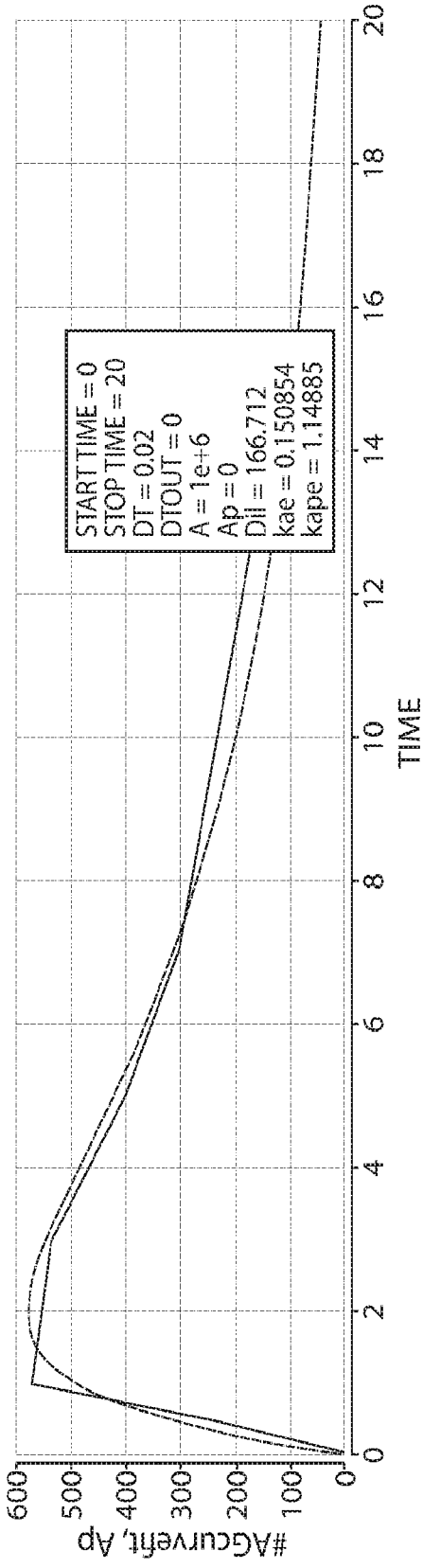

Fig. 18B

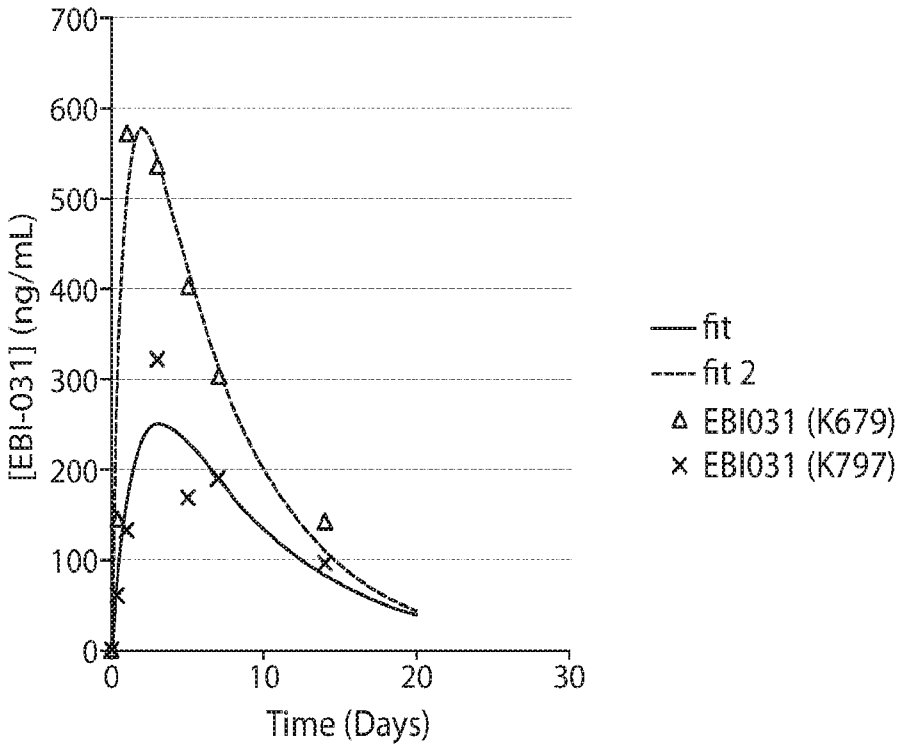
700
600
500
400
300
200
100
0
[EBI-031] (ng/mL)
0
10
20
30
Time (Days)
fit
fit 2
EBI031 (K679)
EBI031 (K797)

Fig. 19

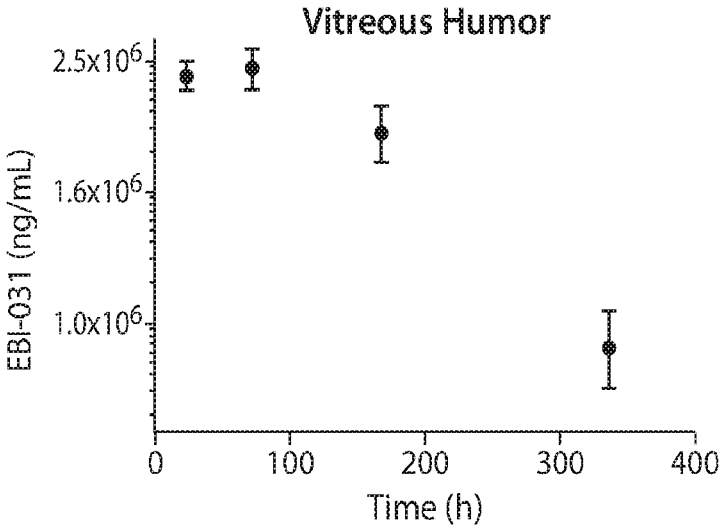

Fig. 20A

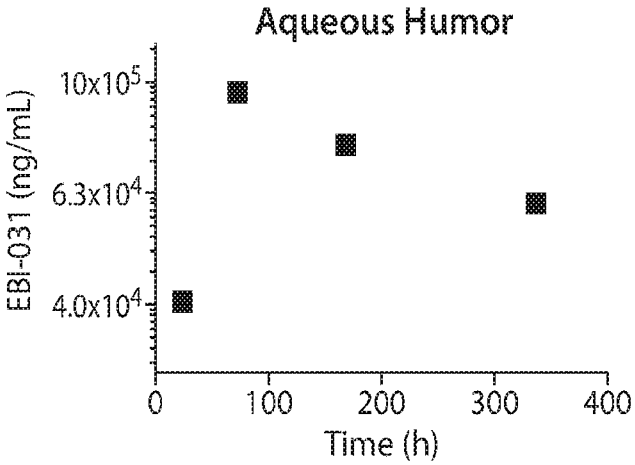

Fig. 20B

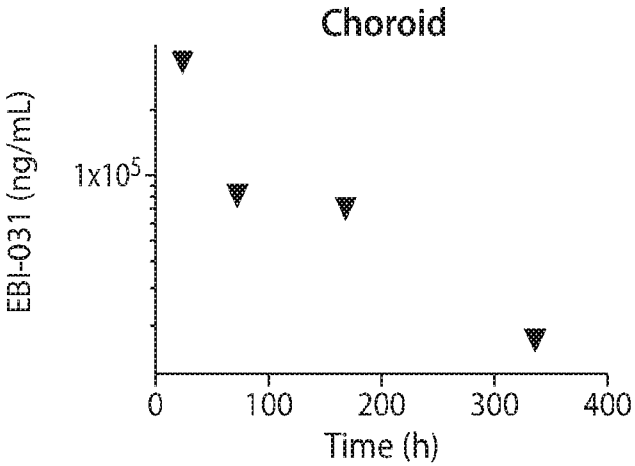

Fig. 20C

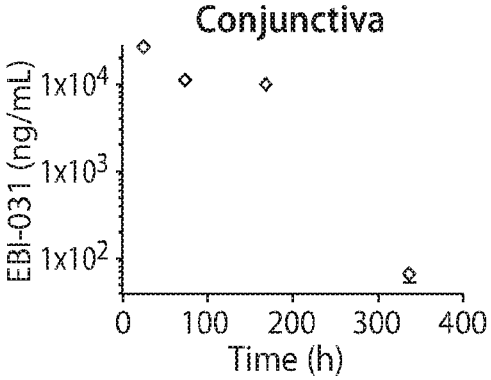

Fig. 20D

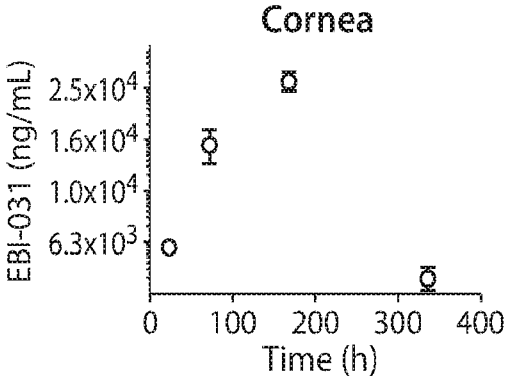

Fig. 20E

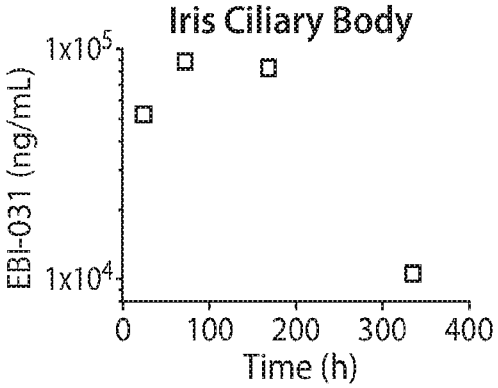

Fig. 20F

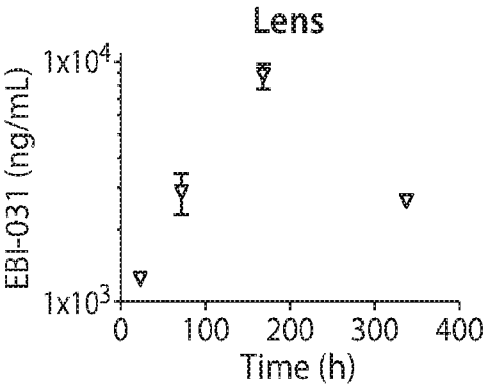

Fig. 20G

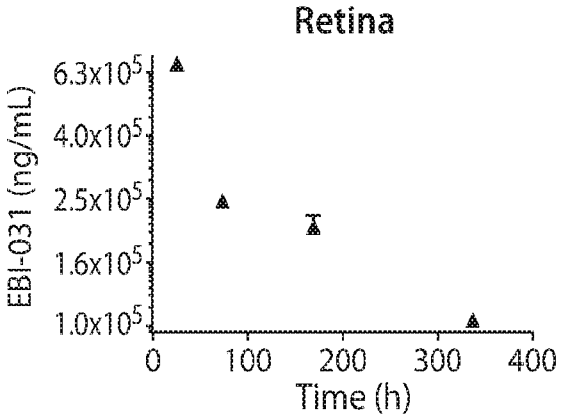

Fig. 20H

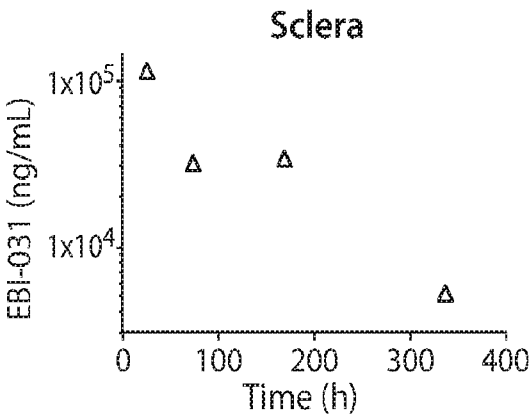

Fig. 20I

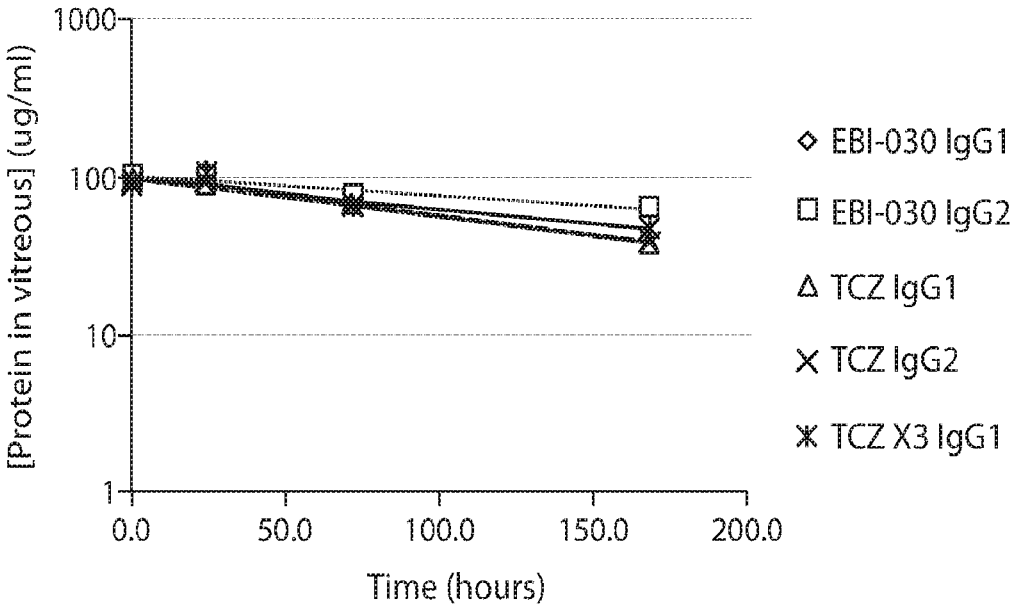

Fig. 21A

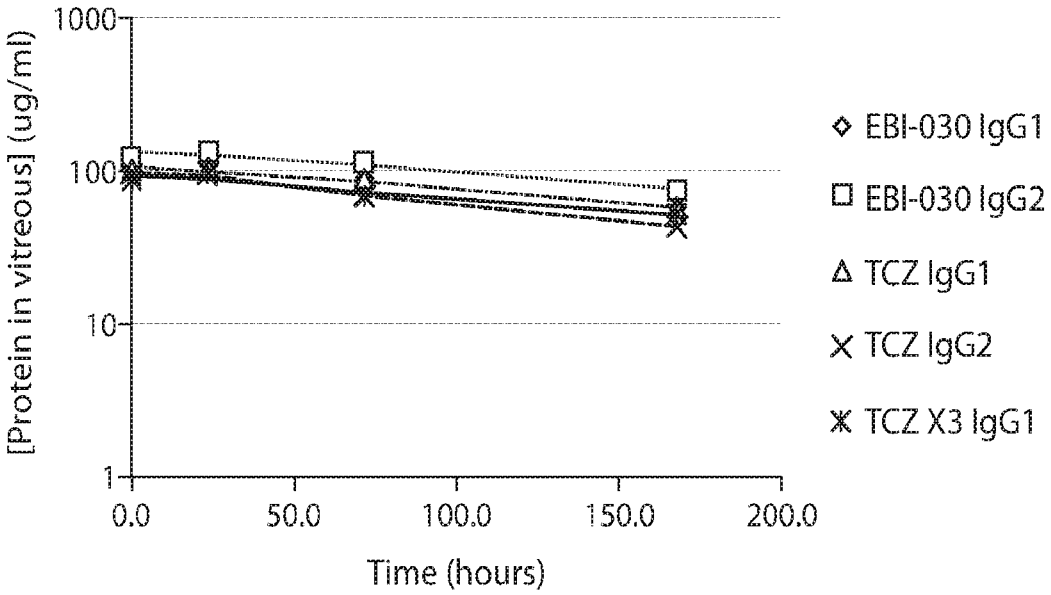

Fig. 21B

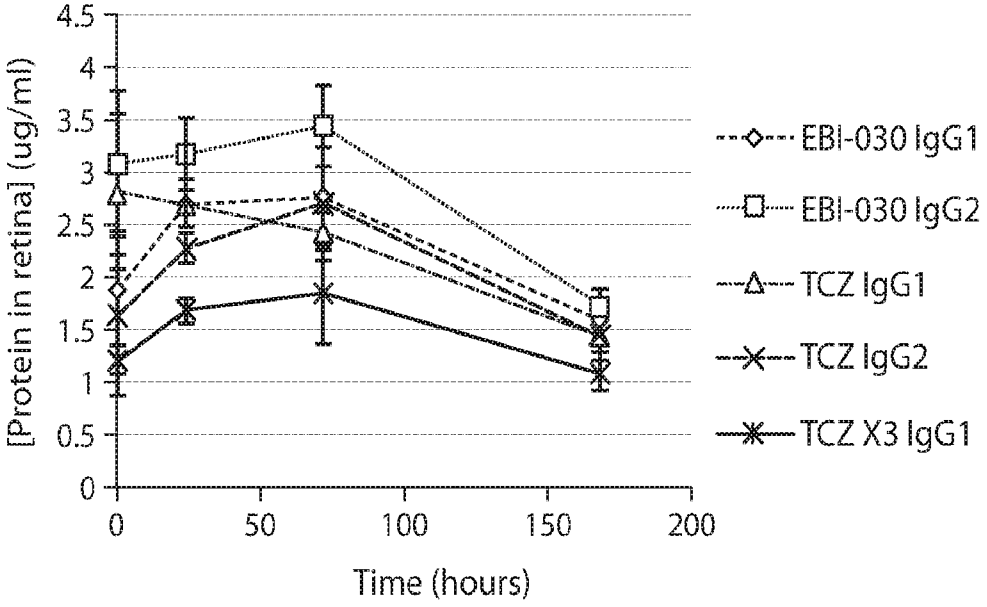
[Protein in retina] (ug/ml)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
50
100
150
200
Time (hours)
EBI-030 IgG1
EBI-030 IgG2
TCZ IgG1
TCZ IgG2
TCZ X3 IgG1

Fig. 22

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/059539

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/24 A61P27/02
ADD. A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal , BIOSIS, EMBASE, Sequence Search , WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Donald J Buchsbaum ET AL: "Comparative Binding and Preclinical Localization and Therapy Studies with Radiolabeled Human Chimeric and Murine 17-1A Monoclonal Antibodies" , CANCER RESEARCH , 1 February 1990 (1990-02-01) , pages 993-999 , XP055251376, Retrieved from the Internet: URL: http://cancerres .aacrjournals.org/content/50/3_Supplement/993s .full.pdf #page=l&view=FitH [retrieved on 2016-02-18] page 995 , right-hand column --- -/- ·	1-8

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" documentwhich may throw doubts on priority claim(s) orwhich is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 March 2016	21/03/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Wagner, Rene

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20 15/059539

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino aoid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [ ] in the form of an Annex C/ST.25 text file.

      [X] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1 (a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [X] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13fer.1 (a)).

      [X] on paper or in the form of an image file (Rule 13ter.1 (b) and Administrative Instructions, Section 7 13).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/059539

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/074905 Al (ELEVEN BIOTHERAPEUTICS INC [US]) 15 May 2014 (2014-05-15) page 55 - page 56 page 24 examples 8, 10 claims 30,31	1-49

X,P	SCHMIDT MICHAEL ET AL: "Optimized intravitreal IL-6 antagonist for the treatment of diabetic macular edema", INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE - I0VS, ASSOCIATION FOR RESEARCH IN VISION AND OPHTHALMOLOGY, US, vol. 56, no. 7, 1 June 2015 (2015-06-01) , page 3488, XP009188261 , ISSN: 0146-0404 abstract	1-49

A	KIM H ET AL: "FcRn receptor-mediated pharmacokinetics of therapeutic IgG in the eye", MOLECULAR VISION , MOLECULAR VISION , US , vol. 15 16 December 2009 (2009-12-16) , pages 2803-2812 , XP002688851 , ISSN: 1090-0535 Retrieved from the Internet: URL: http://www.molvis.org/molvis/vl5/a296 the whole document	1-49

A	M0RDENTI J ET AL: "Comparisons of the intraocular tissue distribution, pharmacokinetics, and safety of 1251-labeled full-length and Fab antibodies in rhesus monkeys following intravitreal administration", TOXICOLOGIC PATHOLOGY, SOCIETY OF TOXICOLOGIC PATHOLOGISTS, LAWRENCE, KS, US, vol. 27, no. 5, 1 September 1999 (1999-09-01) , pages 536-544, XP003034044, ISSN: 0192-6233 , D0I: 10. 1177/019262339902700507 the whole document	1-49

	-/--	

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/059539

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OTTIGER MICHAEL ET AL: "Efficient intraocular penetration of topical anti-TNF-alpha single-chain antibody (ESBA105) to anterior and posterior segment without penetration enhancer", INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE - IOVS, ASSOCIATION FOR RESEARCH IN VISION AND OPHTHALMOLOGY, US, vol. 50, no. 2, 1 February 2009 (2009-02-01), pages 779-786, XP009116265, ISSN: 0146-0404, DOI: 10.1167/IOVS.08-2372 the whole document	1-49
A	US 2009/163699 A1 (CHAMBERLAIN AARON KEITH [US] ET AL) 25 June 2009 (2009-06-25) the whole document	1-49
A	JOSE R. MAYA ET AL: "Emerging Therapies for Noninfectious Uveitis: What May Be Coming to the Clinics", JOURNAL OF OPHTHALMOLOGY, vol. 5, no. 8, 1 January 2014 (2014-01-01), pages 5-7, XP55251091, US ISSN: 2090-004X, DOI: 10.1586/eop.11.19 the whole document	1-49
A	WO 2014/056923 A1 (ASCENDIS PHARMA OPHTHALMOLOGY DIVISION AS [DK]) 17 April 2014 (2014-04-17) the whole document	1-49

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/059539

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014074905 Al	15-05-2014	AU 2013342163 Al	16-04-2015
		CA 2886987 Al	15-05-2014
		CN 104903349 A	09-09-2015
		EP 2917238 Al	16-09-2015
		JP 2016503412 A	04-02-2016
		KR 20150079789 A	08-07-2015
		SG 11201502876R A	29-06-2015
		US 2015125468 Al	07-05-2015
		WO 2014074905 Al	15-05-2014
US 2009163699 Al	25-06-2009	US 2009163699 Al	25-06-2009
		US 2010204454 Al	12-08-2010
		US 2010234571 Al	16-09-2010
		US 2010234572 Al	16-09-2010
		US 2010234573 Al	16-09-2010
		US 2010234574 Al	16-09-2010
		US 2010234575 Al	16-09-2010
		US 2011110928 Al	12-05-2011
		US 2012088905 Al	12-04-2012
		US 2015099863 Al	09-04-2015
		US 2015191533 Al	09-07-2015
WO 2014056923 Al	17-04-2014	AU 2013328782 Al	26-03-2015
		CA 2884910 Al	17-04-2014
		CN 104755103 A	01-07-2015
		EP 2906246 Al	19-08-2015
		JP 2015533132 A	19-11-2015
		KR 20150065891 A	15-06-2015
		SG 11201501914X A	28-05-2015
		US 2015297740 Al	22-10-2015
		WO 2014056923 Al	17-04-2014