OPTIMIZED VARIANTS OF ANTI-VEGF ANTIBODIES

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 21, 2016, is named 50474-110WO3_Sequence_Listing_9_21_16_ST25 and is 41,764 bytes in size.

FIELD OF THE INVENTION

The invention relates generally to anti-VEGF antibodies, as well as compositions that include anti-VEGF antibodies (e.g., antibody conjugates, fusion proteins, and polymeric formulations), with beneficial properties for research, therapeutic, and diagnostic purposes. The invention also relates to methods of identifying antibody variants with improved properties, for example, enhanced binding affinity, stability, and/or expression.

BACKGROUND OF THE INVENTION

Angiogenesis is a tightly-regulated process through which new blood vessels form from pre-existing blood vessels. Although angiogenesis is important during development to ensure adequate blood circulation, many disorders are associated with pathological angiogenesis, such as ocular disorders (e.g., age-related macular degeneration, AMD) and cell proliferative disorders (e.g., cancer). Vascular endothelial growth factor (VEGF) is a clinically-validated driver of angiogenesis and neutralization of VEGF, for example, using an anti-VEGF blocking antibody, can be used to treat disorders associated with pathological angiogenesis.

There remains a need for antibodies, such as anti-VEGF antibodies, with enhanced binding affinity, stability, pharmacokinetics, and/or expression, for example, for use in treating disorders associated with pathological angiogenesis. In particular, there is a need for antibody compositions for long-acting delivery for treatment of ocular disorders (e.g., AMD (e.g., wet AMD), diabetic macular edema (DME), diabetic retinopathy (DR), and retinal vein occlusion (RVO)). In addition, there is an unmet need for improved methods of identifying such antibodies with improved properties (e.g., enhanced binding affinity, stability, pharmacokinetics, and/or expression).

SUMMARY

The present disclosure provides anti-VEGF antibodies, compositions that include anti-VEGF antibodies (e.g., antibody conjugates, fusion proteins, and polymeric formulations), and methods of use thereof, for example, for treatment of disorders associated with pathological angiogenesis (e.g., ocular disorders and cell proliferative disorders). The present disclosure also provides methods of identifying antibody variants with improved properties, for example, enhanced binding affinity, stability, pharmacokinetics, and/or expression.

In a first aspect, there is provided an isolated antibody that specifically binds vascular endothelial growth factor (VEGF), wherein the antibody comprises the following six hypervariable regions (HVRs):

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of $GX_1TPX_2GGX_3X_4X_5YX_6DSVX_7X_8$ (SEQ ID NO: 2), wherein X_1 is Ile or His, X_2 is Ala or Arg, X_3 is Tyr or Lys, X_4 is Thr or Glu, X_5 is Arg, Tyr, Gln, or Glu, X_6 is Ala or Glu, X_7 is Lys or Glu, and X_8 is Gly or Glu;

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of $RASQX_1VSTAVA$ (SEQ ID NO: 4), wherein X_1 is Asp or Arg;

(e) an HVR-L2 comprising the amino acid sequence of $X_1ASFLYS$ (SEQ ID NO: 5), wherein X_1 is Ser or Met; and

(f) an HVR-L3 comprising the amino acid sequence of X_1QGYGX_2PFT (SEQ ID NO: 6), wherein X_1 is Gln, Asn, or Thr and X_2 is Ala, Gln, or Arg.

Disclosed is an isolated antibody that specifically binds vascular endothelial growth factor (VEGF), wherein the antibody comprises the following six hypervariable regions (HVRs): (a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1); (b) an HVR-H2 comprising the amino acid sequence of $GX_1TPX_2GGX_3X_4X_5YX_6DSVX_7X_8$ (SEQ ID NO: 2), wherein X_1 is Ile or His, X_2 is Ala or Arg, X_3 is Tyr or Lys, X_4 is Thr or Glu, X_5 is Arg, Tyr, Gln, or Glu, X_6 is Ala or Glu, X_7 is Lys or Glu, and X_8 is Gly or Glu; (c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3); (d) an HVR-L1 comprising the amino acid sequence of $RASQX_1VSTAVA$ (SEQ ID NO: 4), wherein X_1 is Asp or Arg; (e) an HVR-L2 comprising the amino acid sequence of $X_1ASFLYS$ (SEQ ID NO: 5), wherein X_1 is Ser or Met; and (f) an HVR-L3 comprising the amino acid sequence of X_1QGYGX_2PFT (SEQ ID NO: 6), wherein X_1 is Gln, Asn, or Thr and X_2 is Ala, Asn, Gln, or Arg. In some embodiments, the antibody comprises the following six HVRs: (a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1); (b) an HVR-H2 comprising the amino acid sequence of GITPAGGYTRYADSVKG (SEQ ID NO: 7), GITPAGGYEYYADSVKG (SEQ ID NO: 21), or GITPAGGYEYYADSVEG (SEQ ID NO: 22); (c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3); (d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8); (e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and (f) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10) or QQGYGNPFT (SEQ ID NO: 23).

In some embodiments, the antibody comprises the following six HVRs: (a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1); (b) an HVR-H2 comprising the amino acid sequence of GITPAGGYTRYADSVKG (SEQ ID NO: 7); (c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3); (d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8); (e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and (f) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10). In some embodiments, the antibody further comprises the following heavy chain variable (VH) domain framework regions (FRs): (a) an FR-H1 comprising the amino acid sequence of EVQLVESGGGLVQPGGSLRLSCAASGFTIS (SEQ ID NO: 13); (b) an FR-H2 comprising the amino acid sequence of WVRQAPGKGLEWVA (SEQ ID NO: 14); (c) an FR-H3 comprising the amino acid sequence of RFTISADTSKNTAYLQMRSLRAEDTAVYYCAR (SEQ ID NO: 15); and (d) an FR-H4 comprising the amino acid sequence of WGQGTLVTVSS (SEQ ID NO: 16). In some embodiments, the antibody further comprises the following light chain variable (VL) domain FRs: (a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17); (b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18); (c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19); and (d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

In some embodiments, the antibody comprises the following six HVRs: (a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1); (b) an HVR-H2 comprising the amino acid sequence of GITPAGGYEYYADSVEG (SEQ ID NO: 22); (c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3); (d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8); (e) an HVR-L2 comprising the amino acid sequence of

SASFLYS (SEQ ID NO: 9); and (f) an HVR-L3 comprising the amino acid sequence of QQGYGNPFT (SEQ ID NO: 23). In some embodiments, the antibody further comprises the following VL domain FRs: (a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17); (b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18); (c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO: 24); and (d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

In some embodiments, the antibody comprises the following six HVRs: (a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1); (b) an HVR-H2 comprising the amino acid sequence of GITPAGGYEYYADSVEG (SEQ ID NO: 22); (c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3); (d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8); (e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and (f) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10). In some embodiments, the antibody further comprises the following VL domain FRs: (a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17), DIQMTQSPESLSASVGDEVTITC (SEQ ID NO: 25), or DIQMTQSPSSLSASVGDEVTITC (SEQ ID NO: 26); (b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18) or WYQQKPGEAPKLLIY (SEQ ID NO: 27); (c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19) or GVPSRFSGSGSGTDFTLTIESLQPEDAATYYC (SEQ ID NO: 28); and (d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20). In some embodiments, the antibody further comprises the following VH domain FRs: (a) an FR-H1 comprising the amino acid sequence of EEQLVEEGGGLVQPGESLELSCAASGFEIS (SEQ ID NO: 29) or EEQLVEEGGGLVQPGESLRLSCAASGFEIS (SEQ ID NO: 51); (b) an FR-H2 comprising the amino acid sequence of WVRQEPGEGLEWVA (SEQ ID NO: 30); (c) an FR-H3 comprising the amino acid sequence of RFTISADTSENTAYLQMNELRAEDTAVYYCAR (SEQ ID NO: 31); and (d) an FR-H4 comprising the amino acid sequence of WGQGELVTVSS (SEQ ID NO: 32).

In a second aspect, there is provided an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 11; (b) a VL domain comprising an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12; or (c) a VH domain as in (a) and a VL domain as in (b).

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 11, 40, or 42; (b) a VL domain comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 12, 41, or 46; or (c) a VH domain as in (a) and a VL domain as in (b). In some embodiments, the VH domain further comprises the following FRs: (a) an FR-H1 comprising the amino acid sequence of EVQLVESGGGLVQPGGSLRLSCAASGFTIS (SEQ ID NO: 13); (b) an FR-H2 comprising the amino acid sequence of WVRQAPGKGLEWVA (SEQ ID NO: 14) or WVRQEPGKGLEWVA (SEQ ID NO: 39); (c) an FR-H3 comprising the amino acid sequence of RFTISADTSKNTAYLQMRSLRAEDTAVYYCAR (SEQ ID NO: 15); and (d) an FR-H4 comprising the amino acid sequence of WGQGTLVTVSS (SEQ ID NO: 16). In some embodiments, the VH domain comprises the amino acid sequence of SEQ ID NO: 11. In some embodiments, the VL domain further comprises the following FRs: (a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17) or DIQMTQSPSSLSASVGDRVTIDC (SEQ ID NO:

45); (b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18); (c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19), GVPSRFSGSGSGTDFTLTISSLQPEDSATYYC (SEQ ID NO: 44), or GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC (SEQ ID NO: 54); and (d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20) or FGQGTKVEVK (SEQ ID NO: 55). In some embodiments, the VL domain comprises the amino acid sequence of SEQ ID NO:12.

In a third aspect, there is provided an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 11 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 12.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 40 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 12.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 42 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 12.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 42 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 41.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 33 or 51; (b) a VL domain comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 12, 34, 35, 36, 37, or 38; or (c) a VH domain as in (a) and a VL domain as in (b). In some embodiments, the antibody further comprises the following FRs: (a) an FR-H1 comprising the amino acid sequence of EEQLVEEGGGLVQPGESLELSCAASGFEIS (SEQ ID NO: 29) or EEQLVEEGGGLVQPGESLRLSCAASGFEIS (SEQ ID NO: 52); (b) an FR-H2 comprising the amino acid sequence of WVRQEPGEGLEWVA (SEQ ID NO: 30); (c) an FR-H3 comprising the amino acid sequence of RFTISADTSENTAYLQMNELRAEDTAVYYCAR (SEQ ID NO: 31); and (d) an FR-H4 comprising the amino acid sequence of WGQGELVTVSS (SEQ ID NO: 32). In some embodiments, the VH domain comprises the amino acid sequence of SEQ ID NO: 33. In some embodiments, the VH domain comprises the amino acid sequence of SEQ ID NO: 51. In some embodiments, the antibody further comprises the following FRs: (a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17), DIQMTQSPESLSASVGDEVTITC (SEQ ID NO: 25), or DIQMTQSPSSLSASVGDEVTITC (SEQ ID NO: 26); (b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18) or WYQQKPGEAPKLLIY (SEQ ID NO: 27); (c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19), GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO: 24), or GVPSRFSGSGSGTDFTLTIESLQPEDAATYYC (SEQ ID NO: 28); and (d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20). In some embodiments, the VL domain comprises the amino acid sequence of SEQ ID NO: 34.In some embodiments, the VL domain comprises

the amino acid sequence of SEQ ID NO: 35. In some embodiments, the VL domain comprises the amino acid sequence of SEQ ID NO: 36. In some embodiments, the VL domain comprises the amino acid sequence of SEQ ID NO: 37. In some embodiments, the VL domain comprises the amino acid sequence of SEQ ID NO: 12. In some embodiments, the VL domain comprises the amino acid sequence of SEQ ID NO: 38.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 33 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 38.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 33 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 34.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 33 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 35.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 33 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 36.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 33 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 37.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 33 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 12.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 51 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 38.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 51 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 35.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 51 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 37.

Disclosed is an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 51 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 12.

In a fourth aspect, there is provided an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 48 and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 50.

In a fifth aspect, there is provided an isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 49 and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 50.

In some embodiments of any of the preceding aspects, the antibody is capable of inhibiting the binding of VEGF to a VEGF receptor. In some embodiments, the VEGF receptor is VEGF receptor 1 (Flt-1). In some embodiments, the VEGF receptor is VEGF receptor 2 (KDR).

In some embodiments of any of the preceding aspects, the antibody binds human VEGF (hVEGF) with a Kd of about 2 nM or lower. In some embodiments, the antibody binds hVEGF with a Kd between about 75 pM and about 2 nM. In some embodiments, the antibody binds hVEGF with a Kd between about 75 pM and about 600 pM. In some embodiments, the antibody binds hVEGF with a Kd between about 75 pM and about 500 pM. In some embodiments, the antibody binds hVEGF with a Kd of about 80 pM. In some embodiments, the antibody binds hVEGF with a Kd of about 60 pM.

In some embodiments of any of the preceding aspects, the antibody has a melting temperature (Tm) of greater than about 83.5°C. In some embodiments, the antibody has a Tm of about 85°C to about 91°C. In some embodiments, the antibody has a Tm of about 89°C.

In some embodiments of any of the preceding aspects, the antibody has an isoelectric point (pI) of lower than 8. In some embodiments, the antibody has a pI from about 5 to about 7. In some embodiments, the antibody has a pI of from about 5 to about 6.

In some embodiments of any of the preceding aspects, the antibody is monoclonal, human, humanized, or chimeric.

In some embodiments of any of the preceding aspects, the antibody is an antibody fragment that binds VEGF. In some embodiments, the antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments. In some embodiments, the antibody fragment is an Fab.

In some embodiments of any of the preceding aspects, the antibody is a monospecific antibody. In other embodiments of any of the preceding aspects, the antibody is a multispecific antibody. In some embodiments, the multispecific antibody is a bispecific antibody. In some embodiments, the bispecific antibody binds VEGF and a second biological molecule selected from the group consisting of interleukin 1β (IL-1β); interleukin-6 (IL-6); interleukin-6 receptor (IL-6R); interleukin-13 (IL-13); IL-13 receptor (IL-13R); PDGF; angiopoietin; angiopoietin 2; Tie2; S1P; integrins αvβ3, αvβ5, and α5β1; betacellulin; apelin/APJ; erythropoietin; complement factor D; TNFα; HtrA1; a VEGF receptor; ST-2 receptor; and a protein genetically linked to age-related macular degeneration (AMD) risk. In some embodiments, the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, membrane-bound VEGF-receptor (mbVEGFR), or soluble VEGF receptor (sVEGFR). In some embodiments, the protein genetically linked to AMD risk is selected from the group consisting of complement pathway components C2, factor B, factor H, CFHR3, C3b, C5, C5a, and C3a; HtrA1; ARMS2; TIMP3; HLA; interleukin-8 (IL-8); CX3CR1; TLR3; TLR4; CETP; LIPC, COL10A1; and TNFRSF10A.

Disclosed is a polynucleotide (e.g., an isolated polynucleotide) encoding any of the antibodies as described herein.

In a sixth aspect, there is provided a polynucleotide encoding the antibody as described herein.

In a seventh aspect, there is provided a vector comprising the polynucleotide as described herein. Disclosed is a vector (e.g., an expression vector) comprising the polynucleotide for expressing the antibody.

In an eighth aspect, there is provided a host cell comprising the vector as described herein.

Disclosed are host cells comprising the preceding polynucleotides and/or vectors. In some embodiments, the host cell is a mammalian cell. In some embodiments, the mammalian cell is a 293 cell, a Chinese hamster ovary (CHO) cell, a yeast cell, or a plant cell. In some embodiments, the host cell is a prokaryotic cell. In some embodiments, the prokaryotic cell is *E. coli*.

In a ninth aspect, there is provided a method of producing the antibody as described herein, the method comprising culturing a host cell that comprises the vector as described herein and recovering the antibody. Disclosed is a method of producing any of the antibodies as described herein, the method comprising culturing a host cell that comprises any of the preceding vectors (e.g., expression vectors) in a culture medium. In some embodiments, the method further comprises recovering the antibody from the host cell or the culture medium. In some embodiments, the mammalian cell is a 293 cell, a Chinese hamster ovary (CHO) cell, a yeast cell, or a plant cell. In some embodiments, the host cell is a prokaryotic cell. In some embodiments, the prokaryotic cell is *E. coli*.

In a tenth aspect, there is provided use of the antibody as described herein in the manufacture of a medicament for reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.Disclosed is a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis, comprising administering to the subject an effective amount of any one of the preceding antibodies, thereby reducing or inhibiting angiogenesis in the subject. In some embodiments, the disorder associated with pathological angiogenesis is an ocular disorder or a cell proliferative disorder. In some embodiments, the disorder associated with pathological angiogenesis is an ocular disorder. In some embodiments, the ocular disorder is selected from the group consisting of age-related macular degeneration (AMD), macular degeneration, macular edema, diabetic macular edema (DME) (including focal, non-center DME and diffuse, center-involved DME), retinopathy, diabetic retinopathy (DR) (including proliferative DR (PDR), non-proliferative DR (NPDR), and high-altitude DR), other ischemia-related retinopathies, retinopathy of prematurity (ROP), retinal vein occlusion (RVO) (including central (CRVO) and branched (BRVO) forms), CNV (including myopic CNV), corneal neovascularization, a disease associated with corneal neovascularization, retinal neovascularization, a disease associated with retinal/choroidal neovascularization, pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, familial exudative vitreoretinopathy (FEVR), Coats' disease, Norrie Disease, Osteoporosis-Pseudoglioma Syndrome (OPPG), subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, retinitis pigmentosa (RP), hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, cystoid macular edema (CME), vasculitis, papilloedema, retinitis, conjunctivitis (including infectious conjunctivitis and non-infectious (e.g., allergic) conjunctivitis), Leber congenital amaurosis, uveitis (including infectious and non-infectious uveitis), choroiditis, ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, and Sjögren's disease. In some embodiments, the ocular disorder is AMD, DME, DR, or RVO. In some embodiments, the ocular disorder is AMD. In some embodiments, the AMD is wet AMD. In some embodiments, the disorder associated with pathological angiogenesis is a cell proliferative disorder. In some embodiments, the cell proliferative disorder is a cancer. In some embodiments, the cancer is selected from the group consisting of breast cancer, colorectal cancer, non-small cell lung cancer, non-Hodgkins lymphoma (NHL), renal cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, and multiple myeloma.

In an eleventh aspect, there is provided use of the antibody as described herein in the manufacture of a medicament for treating a disorder associated with pathological angiogenesis.Disclosed is a method for treating a disorder associated with pathological angiogenesis, the method comprising administering an effective amount of any one of the

preceding antibodies to a subject in need of such treatment. In some embodiments, the disorder associated with pathological angiogenesis is an ocular disorder or a cell proliferative disorder. In some embodiments, the disorder associated with pathological angiogenesis is an ocular disorder. In some embodiments, the ocular disorder is selected from the group consisting of age-related macular degeneration (AMD), macular degeneration, macular edema, diabetic macular edema (DME) (including focal, non-center DME and diffuse, center-involved DME), retinopathy, diabetic retinopathy (DR) (including proliferative DR (PDR), non-proliferative DR (NPDR), and high-altitude DR), other ischemia-related retinopathies, retinopathy of prematurity (ROP), retinal vein occlusion (RVO) (including central (CRVO) and branched (BRVO) forms), CNV (including myopic CNV), corneal neovascularization, a disease associated with corneal neovascularization, retinal neovascularization, a disease associated with retinal/choroidal neovascularization, pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, familial exudative vitreoretinopathy (FEVR), Coats' disease, Norrie Disease, Osteoporosis-Pseudoglioma Syndrome (OPPG), subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, retinitis pigmentosa (RP), hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, cystoid macular edema (CME), vasculitis, papilloedema, retinitis, conjunctivitis (including infectious conjunctivitis and non-infectious (e.g., allergic) conjunctivitis), Leber congenital amaurosis, uveitis (including infectious and non-infectious uveitis), choroiditis, ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, and Sjögren's disease. In some embodiments, the ocular disorder is AMD, DME, DR, or RVO. In some embodiments, the ocular disorder is AMD. In some embodiments, the AMD is wet AMD. In some embodiments, the disorder associated with pathological angiogenesis is a cell proliferative disorder. In some embodiments, the cell proliferative disorder is a cancer. In some embodiments, the cancer is selected from the group consisting of breast cancer, colorectal cancer, non-small cell lung cancer, non-Hodgkins lymphoma (NHL), renal cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, and multiple myeloma.

In a twelfth aspect, there is provided use of the antibody as described herein in the manufacture of a medicament for inhibiting vascular permeability in a subject suffering from a disorder associated with undesirable vascular permeability. Disclosed is a method of inhibiting vascular permeability in a subject suffering from a disorder associated with undesirable vascular permeability, the method comprising administering to the subject an effective amount of any one of the preceding antibodies, thereby inhibiting vascular permeability in the subject.

In a thirteenth aspect, there is provided use of the antibody as described herein in the manufacture of a medicament for treating a disorder associated with undesirable vascular permeability in a subject in need thereof. Disclosed is a method of treating a disorder associated with undesirable vascular permeability, the method comprising administering an effective amount of any one of the preceding antibodies to a subject in need of such treatment.

In some embodiments of any of the preceding aspects, the disorder associated with undesirable vascular permeability is selected from the group consisting of edema associated with brain tumors, ascites associated with malignancies, Meigs' syndrome, lung inflammation, nephrotic syndrome, pericardial effusion, pleural effusion, and permeability associated with cardiovascular diseases.

In some embodiments of any of the preceding aspects, the method further comprises administering to the subject an effective amount of a second agent, wherein the second agent is selected from the group consisting of another antibody, a chemotherapeutic agent, a cytotoxic agent, an anti-angiogenic agent, an immunosuppressive agent, a prodrug, a cytokine, a cytokine antagonist, cytotoxic

radiotherapy, a corticosteroid, an anti-emetic, a cancer vaccine, an analgesic, a growth-inhibitory agent, and a compound that binds to a second biological molecule. In some embodiments, the anti-angiogenic agent is a VEGF antagonist. In some embodiments, the VEGF antagonist is an anti-VEGF antibody, an anti-VEGF receptor antibody, a soluble VEGF receptor fusion protein, an aptamer, an anti-VEGF DARPin®, or a VEGFR tyrosine kinase inhibitor. In some embodiments, the anti-VEGF antibody is ranibizumab (LUCENTIS®), RTH-258, or a bispecific anti-VEGF antibody. In some embodiments, the bispecific anti-VEGF antibody is an anti-VEGF/anti-Ang2 antibody. In some embodiments, the anti-VEGF/anti-Ang2 antibody is RG-7716. In some embodiments, the soluble VEGF receptor fusion protein is aflibercept (EYLEA®). In some embodiments, the aptamer is pegaptanib (MACUGEN®). In some embodiments, the anti-VEGF DARPin® is abicipar pegol. In some embodiments, the VEGFR tyrosine kinase inhibitor is selected from the group consisting of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474), 4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171), vatalanib (PTK787), semaxaminib (SU5416), and SUTENT® (sunitinib). In some embodiments, the second biological molecule is selected from the group consisting of IL-1β; IL-6; IL-6R; IL-13; IL-13R; PDGF; angiopoietin; angiopoietin 2; Tie2; S1P; integrins αvβ3, αvβ5, and α5β1; betacellulin; apelin/APJ; erythropoietin; complement factor D; TNFα; HtrA1; a VEGF receptor; ST-2 receptor; and a protein genetically linked to AMD risk. In some embodiments, the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, mbVEGFR, or sVEGFR. In some embodiments, the protein genetically linked to AMD risk is selected from the group consisting of complement pathway components C2, factor B, factor H, CFHR3, C3b, C5, C5a, and C3a; HtrA1; ARMS2; TIMP3; HLA; IL-8; CX3CR1; TLR3; TLR4; CETP; LIPC, COL10A1; and TNFRSF10A. In some embodiments, the compound that binds a second biological molecule is an antibody or antigen-binding fragment thereof. In some embodiments, the antigen-binding antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments.

In some embodiments of any of the preceding aspects, the antibody is administered intravitreally, ocularly, intraocularly, juxtasclerally, subtenonly, superchoroidally, topically, intravenously, intramuscularly, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intrathecally, intranasally, intravaginally, intrarectally, topically, intratumorally, intraperitoneally, peritoneally, intraventricularly, subcutaneously, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraorbitally, orally, transdermally, by inhalation, by injection, by eye drop, by implantation, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, in cremes, or in lipid compositions. In some embodiments, the antibody is administered intravitreally, ocularly, intraocularly, juxtasclerally, subtenonly, superchoroidally, or topically. In some embodiments, the antibody is administered intravitreally by injection. In some embodiments, the antibody is administered topically by eye drop or ointment. In some embodiments, the antibody is administered by a port delivery device. In some embodiments, the subject is a human.

In a fourteenth aspect, there is provided a pharmaceutical composition comprising the antibody as described herein. In some embodiments, the pharmaceutical formulation further comprises a polymer. In some embodiments, the polymer is a biodegradable polymer. In some embodiments, the

a VEGFR tyrosine kinase inhibitor. In some embodiments, the anti-VEGF antibody is ranibizumab (LUCENTIS®), RTH-258, or a bispecific anti-VEGF antibody. In some embodiments, the bispecific anti-VEGF antibody is an anti-VEGF/anti-Ang2 antibody. In some embodiments, the anti-VEGF/anti-Ang2 antibody is RG-7716. In some embodiments, the soluble VEGF receptor fusion protein is aflibercept (EYLEA®). In some embodiments, the aptamer is pegaptanib (MACUGEN®). In some embodiments, the anti-VEGF DARPin® is abicipar pegol. In some embodiments, the VEGFR tyrosine kinase inhibitor is selected from the group consisting of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474), 4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171), vatalanib (PTK787), semaxaminib (SU5416), and SUTENT® (sunitinib). In some embodiments, the second biological molecule is selected from the group consisting of IL-1β; IL-6; IL-6R; IL-13; IL-13R; PDGF; angiopoietin; angiopoietin 2; Tie2; S1P; integrins αvβ3, αvβ5, and α5β1; betacellulin; apelin/APJ; erythropoietin; complement factor D; TNFα; HtrA1; a VEGF receptor; ST-2 receptor; and a protein genetically linked to AMD risk. In some embodiments, the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, mbVEGFR, or sVEGFR. In some embodiments, the protein genetically linked to AMD risk is selected from the group consisting of complement pathway components C2, factor B, factor H, CFHR3, C3b, C5, C5a, and C3a; HtrA1; ARMS2; TIMP3; HLA; IL-8; CX3CR1; TLR3; TLR4; CETP; LIPC, COL10A1; and TNFRSF10A. In some embodiments, the compound that binds a second biological molecule is an antibody or antigen-binding fragment thereof. In some embodiments, the antigen-binding antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments.

In a fifteenth aspect, there is provided the pharmaceutical composition as described herein, for use in the treatment of a disorder associated with pathological angiogenesis or a disorder associated with undesirable vascular permeability in a mammal.

In a sixteenth aspect, there is provided an antibody conjugate comprising (i) the antibody as described herein and (ii) a hydrophilic polymer covalently attached to the antibody. In some embodiments, the hydrophilic polymer is a hyaluronic acid (HA) polymer or a polyethylene glycol (PEG) polymer. In some embodiments, the hydrophilic polymer is an HA polymer. In some embodiments, the HA polymer has a molecular weight of about 1 megadalton (MDa) or lower. In some embodiments of any of the preceding aspects, the HA polymer has a molecular weight between about 25 kDa and about 500 kDa. In some embodiments, the HA polymer has a molecular weight between about 100 kDa and about 250 kDa. In some embodiments, the HA polymer has a molecular weight of about 200 kDa. In some embodiments, the HA polymer is not cross-linked. In some embodiments, the antibody is an antibody fragment that binds VEGF. In some embodiments, the antibody fragment is selected from the group consisting of Fab, Fab-C, Fab', Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments. In some embodiments, the antibody fragment is an Fab, Fab-C, or Fab'. In some embodiments, the antibody conjugate has a hydrodynamic radius between about 10 nm and about 60 nm. In some embodiments, the antibody conjugate has a hydrodynamic radius between about 25 nm and about 35 nm. In some embodiments, the hydrodynamic radius is about 28 nm. In some embodiments, the antibody conjugate has an ocular half-life that is increased relative to a reference antibody that is not covalently attached to the hydrophilic polymer. In some embodiments, the ocular half-life is increased at least about 2-fold relative to the reference antibody. In some embodiments, the ocular half-life is increased at least about 4-fold relative to the reference antibody. In some embodiments, the ocular half-life is a vitreal half-life. In some embodiments, the reference antibody is identical to the antibody of the antibody conjugate.

In a seventeenth aspect, there is provided an antibody conjugate comprising (i) an antibody that specifically binds VEGF and (ii) an HA polymer covalently attached to the antibody, wherein the HA polymer has a molecular weight of 1 MDa or lower. In some embodiments of any of the preceding aspects, the HA polymer has a molecular weight between about 25 kDa and about 500 kDa. In some embodiments, the HA polymer has a molecular weight between about 100 kDa and about 250 kDa. In some embodiments, the HA polymer has a molecular weight of about 200 kDa. In some embodiments, the HA polymer is not cross-linked. In some embodiments, the antibody is an antibody fragment that binds VEGF. In some embodiments, the antibody fragment is selected from the group consisting of Fab, Fab-C, Fab', Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments. In some embodiments, the antibody fragment is an Fab, Fab-C, or Fab'. In some embodiments, the antibody conjugate has a hydrodynamic radius between about 10 nm and about 60 nm. In some embodiments, the antibody conjugate has a hydrodynamic radius between about 25 nm and about 35 nm. In some embodiments, the hydrodynamic radius is about 28 nm. In some embodiments, the antibody conjugate has an ocular half-life that is increased relative to a reference antibody that is not covalently attached to the hydrophilic polymer. In some embodiments, the ocular half-life is increased at least about 2-fold relative to the reference antibody. In some embodiments, the ocular half-life is increased at least about 4-fold relative to the reference antibody. In some embodiments, the ocular half-life is a vitreal half-life. In some embodiments, the reference antibody is identical to the antibody of the antibody conjugate.

In some embodiments of any of the preceding aspects, the antibody is covalently attached to the polymer by a reversible prodrug linker. In some embodiments, the polymer is a hydrogel. In some embodiments, the hydrogel is a PEG-based hydrogel. In some embodiments, the hydrogel is in the shape of a microparticulate bead.

In an eighteenth aspect, there is provided a fusion protein comprising the antibody as described herein covalently attached to an HA binding domain. In some embodiments, the HA binding domain is covalently attached to the heavy chain or the light chain of the antibody. In some embodiments, the HA binding domain is covalently attached to the C-terminus of the heavy chain or to the C-terminus of the light chain. In some embodiments, the HA binding domain is covalently attached to the C-terminus of the heavy chain. In some embodiments, the HA binding domain is covalently attached to the C-terminus of the light chain. In some embodiments, the fusion protein further comprises a linker, the linker being positioned between the antibody and the HA binding domain. In some embodiments, the linker comprises the amino acid sequence of GGGGS (SEQ ID NO: 61). In some embodiments, the linker consists of the amino acid sequence of GGGGS (SEQ ID NO: 61). In some embodiments, the antibody is an antibody fragment that binds VEGF. In some embodiments, the antibody fragment is selected from the group consisting of Fab, Fab-C, Fab', Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments. In some embodiments, the antibody fragment is an Fab. In some embodiments, the HA binding domain is covalently attached to the C-terminus of the CH1 domain of the Fab. In some embodiments, the HA binding protein is covalently attached to the C-terminus of the CL domain of the Fab. In some embodiments, the HA binding domain is selected from the group consisting of a link module, a G1 domain, and a lysine-rich oligopeptide. In some embodiments, the HA binding domain is a link module. In some embodiments, the link module is selected from the group consisting of tumor necrosis factor-stimulated gene 6 (TSG6), CD44, lymphatic vessel

endothelial hyaluronan receptor 1 (LYVE-1), hyaluronan and proteoglycan link protein (HAPLN) 1, HAPLN2, HAPLN3, HAPLN4, aggrecan, brevican, neurocan, phosphacan, versican, CAB61358, KIA0527, stabilin-1, and stabilin-2 link modules. In some embodiments, the link module is a TSG6 link module. In some embodiments, the TSG6 link module is a human TSG6 link module or a rabbit TSG6 link module. In some embodiments, the TSG link module is a human TSG6 link module. In some embodiments, the human TSG6 link module comprises amino acid residues 36-128 of human TSG6.

In some embodiments of any of the preceding aspects, the fusion protein further comprises at least one additional HA binding domain. In some embodiments, the at least one additional HA binding domain is covalently attached to the heavy chain or the light chain of the antibody. In some embodiments, the at least one additional HA binding protein is linked to the antibody by a linker. In some embodiments, the linker comprises the amino acid sequence of GGGGS (SEQ ID NO: 61). In some embodiments, the linker consists of the amino acid sequence of GGGGS (SEQ ID NO: 61). In some embodiments, a first HA binding domain is covalently attached to the heavy chain and a second HA binding domain is covalently attached to the light chain. In some embodiments, the first HA binding domain is linked to the C-terminus of the heavy chain and the second HA binding domain is linked to the C-terminus of the light chain. In some embodiments, the at least one additional HA binding protein is selected from the group consisting of a link module, a G1 domain, and a lysine-rich oligopeptide. In some embodiments, the at least one additional HA binding protein is a link module. In some embodiments, the link module is a TSG6 link module. In some embodiments, the TSG6 link module is a human TSG6 link module or a rabbit TSG6 link module. In some embodiments, the TSG6 link module is a human TSG6 link module. In some embodiments, the human TSG6 link module comprises amino acid residues 36-128 of human TSG6.

In some embodiments of any of the preceding aspects, the fusion protein specifically binds to VEGF and HA. In some embodiments, the fusion protein binds HA with a Kd of about 2 μM or lower. In some embodiments, the fusion protein binds HA with a Kd between about 1 nM and about 500 nM. In some embodiments, the fusion protein binds HA with a Kd between about 1 nM and about 50 nM. In some embodiments, the fusion protein binds HA with a Kd of about 10 nM.

In some embodiments of any of the preceding aspects, the fusion protein has an ocular half-life that is increased relative to a reference antibody that is not covalently attached to a HA binding domain. In some embodiments, the ocular half-life is increased at least about 2-fold relative to the reference antibody. In some embodiments, the ocular half-life is increased at least about 4-fold relative to the reference antibody. In some embodiments, the ocular half-life is a vitreal half-life. In some embodiments, the reference antibody is identical to the antibody of the fusion protein.

In a nineteenth aspect, there is provided use of the antibody conjugate as described herein in the manufacture of a medicament for reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis. Disclosed is a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis, comprising administering to the subject an effective amount of any one of the preceding antibody conjugates, thereby reducing or inhibiting angiogenesis in the subject.

In a twentieth aspect, there is provided use of the antibody conjugate as described herein in the manufacture of a medicament for treating a disorder associated with pathological angiogenesis in a subject in need of such treatment. Disclosed is a method for treating a disorder associated with pathological angiogenesis, the method comprising administering an effective amount of any one of the preceding antibody conjugates to a subject in need of such treatment.

In a twenty-first aspect, there is provided use of the fusion protein as described herein in the manufacture of a medicament for reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis. Disclosed is a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis, comprising administering to the subject an effective amount of any one of the preceding fusion proteins, thereby reducing or inhibiting angiogenesis in the subject.

In a twenty-second aspect, there is provided use of the fusion protein as described herein in the manufacture of a medicament for treating a disorder associated with pathological angiogenesis in a subject in need of such treatment. Disclosed is a method for treating a disorder associated with pathological angiogenesis, the method comprising administering an effective amount of any one of the preceding fusion proteins to a subject in need of such treatment.

In some embodiments of any of the preceding aspects, the disorder associated with pathological angiogenesis is an ocular disorder. In some embodiments, the ocular disorder is selected from the group consisting of AMD, macular degeneration, macular edema, DME (including focal, non-center DME and diffuse, center-involved DME), retinopathy, DR (including PDR, NPDR, and high-altitude DR), other ischemia-related retinopathies, ROP, RVO (including CRVO and BRVO forms), CNV (including myopic CNV), corneal neovascularization, a disease associated with corneal neovascularization, retinal neovascularization, a disease associated with retinal/choroidal neovascularization, pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, FEVR, Coats' disease, Norrie Disease, OPPG, subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, RP, hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, CME, vasculitis, papilloedema, retinitis, conjunctivitis (including infectious conjunctivitis and non-infectious (e.g., allergic) conjunctivitis), Leber congenital amaurosis, uveitis (including infectious and non-infectious uveitis), choroiditis, ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, and Sjögren's disease. In some embodiments, the ocular disorder is AMD, DME, DR, or RVO. In some embodiments, the ocular disorder is AMD. In some embodiments, the AMD is wet AMD.

In some embodiments of any of the preceding aspects, the method further comprises administering to the subject an effective amount of a second agent, wherein the second agent is selected from the group consisting of another antibody, an anti-angiogenic agent, a cytokine, a cytokine antagonist, a corticosteroid, an analgesic, and a compound that binds to a second biological molecule. In some embodiments, the anti-angiogenic agent is a VEGF antagonist. In some embodiments, the VEGF antagonist is an anti-VEGF antibody, an anti-VEGF receptor antibody, a soluble VEGF receptor fusion protein, an aptamer, an anti-VEGF DARPin®, or a VEGFR tyrosine kinase inhibitor. In some embodiments, the anti-VEGF antibody is ranibizumab (LUCENTIS®), RTH-258, or a bispecific anti-VEGF antibody. In some embodiments, the bispecific anti-VEGF antibody is an anti-VEGF/anti-Ang2 antibody. In some embodiments, the anti-VEGF/anti-Ang2 antibody is RG-7716. In some embodiments, the soluble VEGF receptor fusion protein is aflibercept (EYLEA®). In some embodiments, the aptamer is pegaptanib (MACUGEN®). In some embodiments, the anti-VEGF DARPin® is abicipar pegol. In some embodiments, the VEGFR tyrosine kinase inhibitor is selected from the group consisting of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474), 4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171), vatalanib (PTK787), semaxaminib (SU5416), and SUTENT® (sunitinib). In some embodiments, the second biological molecule is selected from the group consisting of IL-1β; IL-6; IL-6R; IL-13; IL-13R; PDGF; angiopoietin;

angiopoietin 2; Tie2; S1P; integrins αvβ3, αvβ5, and α5β1; betacellulin; apelin/APJ; erythropoietin; complement factor D; TNFα; HtrA1; a VEGF receptor; ST-2 receptor; and a protein genetically linked to AMD risk. In some embodiments, the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, mbVEGFR, or sVEGFR. In some embodiments, the protein genetically linked to AMD risk is selected from the group consisting of complement pathway components C2, factor B, factor H, CFHR3, C3b, C5, C5a, and C3a; HtrA1; ARMS2; TIMP3; HLA; IL-8; CX3CR1; TLR3; TLR4; CETP; LIPC, COL10A1; and TNFRSF10A. In some embodiments, the compound that binds a second biological molecule is an antibody or antigen-binding fragment thereof. In some embodiments, the antigen-binding antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments.

In some embodiments of any of the preceding aspects, the antibody conjugate is administered intravitreally, ocularly, intraocularly, juxtasclerally, subtenonly, superchoroidally, topically, intravenously, intramuscularly, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intrathecally, intranasally, intravaginally, intrarectally, topically, intratumorally, intraperitoneally, peritoneally, intraventricularly, subcutaneously, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraorbitally, orally, transdermally, by inhalation, by injection, by eye drop, by implantation, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, in cremes, or in lipid compositions. In some embodiments, the antibody conjugate is administered intravitreally, ocularly, intraocularly, juxtasclerally, subtenonly, superchoroidally, or topically. In some embodiments, the antibody conjugate is administered intravitreally by injection. In some embodiments, the antibody conjugate is administered topically by eye drop or ointment. In some embodiments, the antibody conjugate is administered by a port delivery device.

In some embodiments of any of the preceding aspects, the fusion protein is administered intravitreally, ocularly, intraocularly, juxtasclerally, subtenonly, superchoroidally, topically, intravenously, intramuscularly, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intrathecally, intranasally, intravaginally, intrarectally, topically, intratumorally, intraperitoneally, peritoneally, intraventricularly, subcutaneously, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraorbitally, orally, transdermally, by inhalation, by injection, by eye drop, by implantation, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, in cremes, or in lipid compositions. In some embodiments, the fusion protein is administered intravitreally, ocularly, intraocularly, juxtasclerally, subtenonly, superchoroidally, or topically. In some embodiments, the fusion protein is administered intravitreally by injection. In some embodiments, the fusion protein is administered topically by eye drop or ointment. In some embodiments, the fusion protein is administered by a port delivery device. In some embodiments, the subject is a human.

In a twenty-third aspect, there is provided a pharmaceutical composition comprising the antibody conjugate as described herein.

In a twenty-fourth aspect, there is provided a pharmaceutical composition comprising the fusion protein as described herein.

biological molecule is an antibody or antigen-binding fragment thereof. In some embodiments, the antigen-binding antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments.

In a twenty-fifth aspect, there is provided a method of identifying an amino acid residue alteration that confers enhanced binding of an antibody to a target molecule, the method comprising: (a) providing a display library comprising nucleic acids encoding candidate antibody variants, wherein each candidate antibody variant comprises an amino acid residue alteration in the VH or the VL compared to a reference antibody, and wherein amino acid residue alterations at every position of the VH or VL are present in the display library; (b) sorting the display library based on binding of the candidate antibody variants to the target molecule to form a sorted library, wherein the sorted library comprises candidate antibody variants with enhanced binding to the target molecule compared to the reference antibody; and (c) comparing the frequency at which each amino acid residue alteration is present in the display library and in the sorted library as determined by massively parallel sequencing, thereby determining whether each amino acid residue alteration is enriched in the sorted library compared to the display library, whereby the amino acid residue alteration is identified as conferring enhanced binding to the target molecule if it is enriched in the sorted library compared to the display library.

In a twenty-sixth aspect, there is provided a method of identifying an amino acid residue alteration that confers enhanced stability to an antibody, the method comprising: (a) providing a display library comprising nucleic acids encoding candidate antibody variants, wherein each candidate antibody variant comprises an amino acid residue alteration in the VH or the VL compared to a reference antibody, and wherein amino acid residue alterations at every position of the VH or VL are present in the display library; (b) sorting the display library based on binding of the candidate antibody variants to the target molecule to form a sorted library, wherein the sorted library comprises candidate antibody variants with enhanced stability compared to the reference antibody; and (c) comparing the frequency at which each amino acid residue alteration is present in the display library and in the sorted library as determined by massively parallel sequencing, thereby determining whether each amino acid residue alteration is enriched in the sorted library compared to the display library, whereby the amino acid residue alteration is identified as conferring enhanced stability to the antibody if it is enriched in the sorted library compared to the display library.

In some embodiments of any of the preceding aspects, the method further comprises determining the frequency at which each amino acid alteration is present in the display library and the sorted library by massively parallel sequencing following step (b).

In some embodiments of any of the preceding aspects, the amino acid residue alteration is enriched at least 2-fold in the sorted library compared to the display library.

In some embodiments of any of the preceding aspects, the display library is selected from the group consisting of a phage display library, a bacterial display library, a yeast display library, a mammalian display library, a ribosome display library, and an mRNA display library. In some embodiments, the display library is a phage display library.

In some embodiments of any of the preceding aspects, the amino acid residue alteration is encoded by a degenerate codon set. In some embodiments, the degenerate codon set is an *NNK* or an

NNS codon set, wherein *N* is A, C, G, or T; *K* is G or T; and *S* is C or G. In some embodiments, the degenerate codon set is an *NNK* codon set.

In some embodiments of any of the preceding aspects, the sorting of step (b) comprises contacting the display library with an immobilized target molecule or epitope.

In some embodiments of any of the preceding aspects, the sorting of step (b) comprises contacting the display library with a soluble target molecule or epitope.

In some embodiments of any of the preceding aspects, the display library comprises at least $1x10^6$ candidate antibody variants. In some embodiments, the display library comprises at least $1x10^8$ antibody variants. In some embodiments, the display library comprises at least $1x10^9$ antibody variants.

In some embodiments of any of the preceding aspects, the massively parallel sequencing comprises deep sequencing, ultra-deep sequencing, and/or next-generation sequencing.

In some embodiments of any of the preceding aspects, the antibody is a monoclonal antibody.

In some embodiments of any of the preceding aspects, the antibody is an IgG antibody.

In some embodiments of any of the preceding aspects, the antibody is an antibody fragment. In some embodiments, antibody fragment is selected from the group consisting of Fab, scFv, Fv, Fab', Fab'-SH, $F(ab')_2$, and diabody. In some embodiments, the antibody fragment is an Fab.

In some embodiments of any of the preceding aspects, the method further comprises generating an antibody that comprises an amino acid residue alteration identified by the steps of the method.

In some embodiments, the antibody as desbribed herein can be used in the manufacture of a medicament for reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

In some embodiments, the antibody as desbribed herein can be used in the manufacture of a medicament for treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

In some embodiments, the antibody as desbribed herein can be used in the manufacture of a medicament for inhibiting vascular permeability in a subject suffering from a disorder associated with undesirable vascular permeability.

In a twenty-seventh aspect, there is provided the antibody as described herein for use in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

In a twenth-eighth aspect, there is provided the antibody as described herein for use in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

In a twenty-ninth aspect, there is provided the antibody as described herein for use in a method of inhibiting vascular permeability in a subject suffering from a disorder associated with undesirable vascular permeability.

In a thirtieth aspect, there is provided a composition comprising the antibody as described herein for use in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

In a thirty-first aspect, there is provided a composition comprising the antibody as described herein for use in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

In a thirty-second aspect, there is provided a composition comprising the antibody as described herein for use in a method of inhibiting vascular permeability in a subject suffering from a disorder associated with undesirable vascular permeability.

In a thirty-third aspect, there is provided the antibody conjugate as described herein for use in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis. In some embodiments, the antibody conjugate as desbribed herein can be used in the manufacture of a medicament for reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

In a thirty-fourth aspect, there is provided the antibody conjugate as described herein for use in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment. In some embodiments, the antibody conjugate as desbribed herein can be used in the manufacture of a medicament for treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

In some embodiments, the antibody conjugate as described herein can be used in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

In some embodiments, the antibody conjugate as described herein can be used in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

In a thirty-fifth aspect, there is provided a composition comprising the antibody conjugate as described herein for use in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

In a thirty-sixth aspect, there is provided a composition comprising the antibody conjugate as described herein for use in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

In some embodiments, the fusion protein as described herein can be used in the manufacture of a medicament for reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

In some embodiments, the fusion protein as described herein can be used in the manufacture of a medicament for treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

In a thirty-seventh aspect, there is provided the fusion protein as described herein for use in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis. In some embodiments, the fusion protein as described herein can be used in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

In a thirty-eighth aspect, there is provided the fusion protein as described herein for use in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment. In some embodiments, the fusion protein as described herein can be used in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

In a thirty-ninth aspect, there is provided a composition comprising the fusion protein as described herein for use in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

In a fourtieth aspect, there is provided a composition comprising the fusion protein as described herein for use in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

It is to be understood that any of the embodiments described above with respect to methods of treatment (e.g., with respect to antibody properties, additional therapeutic agents, disorders associated with pathological angiogenesis (e.g., ocular disorders such as AMD, DME, DR, or RVO), administration

CLAIMS

What is claimed is:

1. An isolated antibody that specifically binds vascular endothelial growth factor (VEGF), wherein the antibody comprises the following six hypervariable regions (HVRs):

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of $GX_1TPX_2GGX_3X_4X_5YX_6DSVX_7X_8$ (SEQ ID NO: 2), wherein X_1 is Ile or His, X_2 is Ala or Arg, X_3 is Tyr or Lys, X_4 is Thr or Glu, X_5 is Arg, Tyr, Gln, or Glu, X_6 is Ala or Glu, X_7 is Lys or Glu, and X_8 is Gly or Glu;

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of $RASQX_1VSTAVA$ (SEQ ID NO: 4), wherein X_1 is Asp or Arg;

(e) an HVR-L2 comprising the amino acid sequence of $X_1ASFLYS$ (SEQ ID NO: 5), wherein X_1 is Ser or Met; and

(f) an HVR-L3 comprising the amino acid sequence of X_1QGYGX_2PFT (SEQ ID NO: 6), wherein X_1 is Gln, Asn, or Thr and X_2 is Ala, Gln, or Arg.

2. The antibody of claim 1, wherein (I) the antibody comprises the following six HVRs:

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of GITPAGGYTRYADSVKG (SEQ ID NO: 7), GITPAGGYEYYADSVKG (SEQ ID NO: 21), or GITPAGGYEYYADSVEG (SEQ ID NO: 22);

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(f) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10) or QQGYGNPFT (SEQ ID NO: 23); wherein optionally (II)

the antibody is selected from a group consisting of

(i) an antibody comprising the following six HVRs:

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of GITPAGGYTRYADSVKG (SEQ ID NO: 7);

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(f) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10);

(ii) an antibody comprising the following six HVRs:

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of GITPAGGYEYYADSVEG (SEQ ID NO: 22);

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);
(e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and
(f) an HVR-L3 comprising the amino acid sequence of QQGYGNPFT (SEQ ID NO: 23); and

(iii) an antibody comprising the following six HVRs:
(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);
(b) an HVR-H2 comprising the amino acid sequence of GITPAGGYEYYADSVEG (SEQ ID NO: 22);
(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);
(d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);
(e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and
(f) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10).

3. The antibody of any one of claims 12(I) and 2(II)(i), wherein the antibody further comprises the following heavy chain variable (VH) domain framework regions (FRs):
(a) an FR-H1 comprising the amino acid sequence of EVQLVESGGGLVQPGGSLRLSCAASGFTIS (SEQ ID NO: 13);
(b) an FR-H2 comprising the amino acid sequence of WVRQAPGKGLEWVA (SEQ ID NO: 14);
(c) an FR-H3 comprising the amino acid sequence of RFTISADTSKNTAYLQMRSLRAEDTAVYYCAR (SEQ ID NO: 15); and
(d) an FR-H4 comprising the amino acid sequence of WGQGTLVTVSS (SEQ ID NO: 16); wherein optionally

the antibody further comprises the following light chain variable (VL) domain FRs:
(a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17);
(b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18);
(c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19); and
(d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

4. The antibody of claim 2(II)(ii), wherein the antibody further comprises the following VL domain FRs:
(a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17);
(b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18);
(c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO: 24); and
(d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

5. The antibody of claim 2(II)(iii), wherein the antibody further comprises the following VL domain FRs:

(a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17), DIQMTQSPESLSASVGDEVTITC (SEQ ID NO: 25), or DIQMTQSPSSLSASVGDEVTITC (SEQ ID NO: 26);

(b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18) or WYQQKPGEAPKLLIY (SEQ ID NO: 27);

(c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19) or GVPSRFSGSGSGTDFTLTIESLQPEDAATYYC (SEQ ID NO: 28); and

(d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

6. The antibody of any one of claims 2(II)(ii)-2(II)(iii), and 4-5, wherein the antibody further comprises the following VH domain FRs:

(a) an FR-H1 comprising the amino acid sequence of EEQLVEEGGGLVQPGESLELSCAASGFEIS (SEQ ID NO: 29) or EEQLVEEGGGLVQPGESLRLSCAASGFEIS (SEQ ID NO: 51);

(b) an FR-H2 comprising the amino acid sequence of WVRQEPGEGLEWVA (SEQ ID NO: 30);

(c) an FR-H3 comprising the amino acid sequence of RFTISADTSENTAYLQMNELRAEDTAVYYCAR (SEQ ID NO: 31); and

(d) an FR-H4 comprising the amino acid sequence of WGQGELVTVSS (SEQ ID NO: 32).

7. An isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 11; (b) a VL domain comprising an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12; or (c) a VH domain as in (a) and a VL domain as in (b).

8. The antibody of claim 7, wherein the VH domain further comprises the following FRs:

(a) an FR-H1 comprising the amino acid sequence of EVQLVESGGGLVQPGGSLRLSCAASGFTIS (SEQ ID NO: 13);

(b) an FR-H2 comprising the amino acid sequence of WVRQAPGKGLEWVA (SEQ ID NO: 14) or WVRQEPGKGLEWVA (SEQ ID NO: 39);

(c) an FR-H3 comprising the amino acid sequence of RFTISADTSKNTAYLQMRSLRAEDTAVYYCAR (SEQ ID NO: 15); and

(d) an FR-H4 comprising the amino acid sequence of WGQGTLVTVSS (SEQ ID NO: 16); wherein optionally

the VH domain comprises the amino acid sequence of SEQ ID NO: 11; and wherein optionally

the VL domain further comprises the following FRs:

(a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17) or DIQMTQSPSSLSASVGDRVTIDC (SEQ ID NO: 45);

(b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18);

(c) an FR-L3 comprising the amino acid sequence of
GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19)
GVPSRFSGSGSGTDFTLTISSLQPEDSATYYC (SEQ ID NO: 44), or
GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC (SEQ ID NO: 54); and

(d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20) or FGQGTKVEVK (SEQ ID NO: 55), wherein optionally

the VL domain comprises the amino acid sequence of SEQ ID NO: 12.

9. An isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 11 and (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 12.

10. An isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 48 and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 50.

11. An isolated antibody that specifically binds VEGF, wherein the antibody comprises (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 49 and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 50.

12. The antibody of any one of claims 1-11, wherein the antibody is capable of inhibiting the binding of VEGF to a VEGF receptor; wherein optionally

the VEGF receptor is VEGF receptor 1 (Flt-1), or

VEGF receptor 2 (KDR); wherein optionally

the antibody binds human VEGF (hVEGF) with a Kd of about 2 nM or lower, wherein optionally

the antibody binds hVEGF with a Kd between about 75 pM and about 2 nM, wherein optionally

the antibody binds hVEGF with a Kd between about 75 pM and about 600 pM, wherein optionally

the antibody binds hVEGF with a Kd between about 75 pM and about 500 pM, and wherein optionally

the antibody binds hVEGF with a Kd of about 60 pM.

13. The antibody of any one of claims 1-3, 7-9 and 10-12, wherein the antibody has a melting temperature (Tm) of greater than about 83.5°C; wherein optionally

the antibody has a Tm of about 85°C to about 91°C, and wherein optionally

the antibody has a Tm of about 89°C.

14. The antibody of any one of claims 1, 2(I), 2(II)(ii)-2(II)(iii), 4-6, and 12, wherein the antibody has an isoelectric point (pI) of lower than 8; wherein optionally

the antibody has a pI from about 5 to about 7, and wherein optionally

the antibody has a pI of from about 5 to about 6.

15. The antibody of any one of claims 1-14, wherein the antibody is monoclonal, human, humanized, or chimeric; wherein optionally

the antibody is an antibody fragment that binds VEGF, wherein optionally

the antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments, wherein optionally

the antibody fragment is an Fab; wherein optionally

the antibody is a monospecific antibody or

a multispecific antibody, wherein optionally

the multispecific antibody is a bispecific antibody, wherein optionally

the bispecific antibody binds VEGF and a second biological molecule selected from the group consisting of IL-1β; interleukin-6 (IL-6); interleukin-6 receptor (IL-6R); interleukin-13 (IL-13); IL-13 receptor (IL-13R); PDGF; angiopoietin; angiopoietin 2; Tie2; S1P; integrins αvβ3, αvβ5, and α5β1; betacellulin; apelin/APJ; erythropoietin; complement factor D; TNFα; HtrA1; a VEGF receptor; ST-2 receptor; and a protein genetically linked to age-related macular degeneration (AMD) risk, wherein optionally

the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, membrane-bound VEGF-receptor (mbVEGFR), or soluble VEGF receptor (sVEGFR), or

the protein genetically linked to AMD risk is selected from the group consisting of complement pathway components C2, factor B, factor H, CFHR3, C3b, C5, C5a, and C3a; HtrA1; ARMS2; TIMP3; HLA; interleukin-8 (IL-8); CX3CR1; TLR3; TLR4; CETP; LIPC, COL10A1; and TNFRSF10A.

16. A polynucleotide encoding the antibody of any one of claims 1-15.

17. A vector comprising the polynucleotide of claim 16.

18. A host cell comprising the vector of claim 17.

19. A method of producing the antibody of any one of claims 1-15, the method comprising culturing a host cell that comprises the vector of claim 17 and recovering the antibody.

20. The method of claim 19, wherein the host cell is prokaryotic, wherein optionally

the host cell is *Escherichia coli;* or

the host cell is eukaryotic, wherein optionally

the host cell is a 293 cell, a CHO cell, a yeast cell, or a plant cell.

21. Use of the antibody of any one of claims 1-15 in the manufacture of a medicament for reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

22. Use of the antibody of any one of claims 1-15 in the manufacture of a medicament for treating a disorder associated with pathological angiogenesis.

23. The use of claim 21 or 22, wherein (i) the disorder associated with pathological angiogenesis is an ocular disorder or a cell proliferative disorder; wherein optionally

(ii) the disorder associated with pathological angiogenesis is an ocular disorder; wherein optionally

(iii) the ocular disorder is selected from the group consisting of age-related macular degeneration (AMD), macular degeneration, macular edema, diabetic macular edema (DME) (including focal, non-center DME and diffuse, center-involved DME), retinopathy, diabetic (DR) (including proliferative DR (PDR), non-proliferative DR (NPDR), and high-altitude DR), other ischemia-related retinopathies, retinopathy of prematurity (ROP), retinal vein occlusion (RVO) (including central RVO (CRVO) and

branched RVO (BRVO) forms), CNV (including myopic CNV), corneal neovascularization, a disease associated with corneal neovascularization, retinal neovascularization, a disease associated with retinal/choroidal neovascularization, pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, familial exudative vitreoretinopathy (FEVR), Coats' disease, Norrie Disease, Osteoporosis-Pseudoglioma Syndrome (OPPG), subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, retinitis pigmentosa (RP), hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, cystoid macular edema (CME), vasculitis, papilloedema, retinitis, conjunctivitis (including infectious conjunctivitis and non-infectious (e.g., allergic) conjunctivitis), Leber congenital amaurosis, uveitis (including infectious and non-infectious uveitis), choroiditis, ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, and Sjögren's disease; and wherein optionally

(iv) the ocular disorder is AMD, DME, DR, or RVO.

24. The use of claim 23(iii) or 23(iv), wherein the ocular disorder is AMD; wherein optionally

the AMD is wet AMD.

25. The use of claim 23(i), wherein the disorder associated with pathological angiogenesis is a cell proliferative disorder; wherein optionally

the cell proliferative disorder is a cancer; and wherein optionally

the cancer is selected from the group consisting of breast cancer, colorectal cancer, non-small cell lung cancer, non-Hodgkins lymphoma (NHL), renal cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, and multiple myeloma.

26. Use of the antibody of any one of claims 1-15 in the manufacture of a medicament for inhibiting vascular permeability in a subject suffering from a disorder associated with undesirable vascular permeability.

27. Use of the antibody of any one of claims 1-15 in the manufacture of a medicament for treating a disorder associated with undesirable vascular permeability in a subject in need thereof.

28. The use of claim 26 or 27, wherein the disorder associated with undesirable vascular permeability is selected from the group consisting of edema associated with brain tumors, ascites associated with malignancies, Meigs' syndrome, lung inflammation, nephrotic syndrome, pericardial effusion, pleural effusion, and permeability associated with cardiovascular diseases.

29. The use of any one of claims 21-28, wherein (i) the medicament is to be admininstered with a second agent, wherein the second agent is selected from the group consisting of another antibody, a chemotherapeutic agent, a cytotoxic agent, an anti-angiogenic agent, an immunosuppressive agent, a prodrug, a cytokine, a cytokine antagonist, cytotoxic radiotherapy, a corticosteroid, an anti-emetic, a cancer vaccine, an analgesic, a growth-inhibitory agent, and a compound that binds to a second biological molecule; wherein optionally (ii)

the anti-angiogenic agent is a VEGF antagonist; wherein optionally (iii)

the VEGF antagonist is an anti-VEGF antibody, an anti-VEGF receptor antibody, a soluble VEGF receptor fusion protein, an aptamer, an anti-VEGF DARPin®, or a VEGFR tyrosine kinase inhibitor; wherein optionally (iv)

the anti-VEGF antibody is ranibizumab (LUCENTIS®), RTH-258, or a bispecific anti-VEGF antibody, wherein optionally the bispecific anti-VEGF antibody is an anti-VEGF/anti-Ang2 antibody, and wherein optionally the anti-VEGF/anti-Ang2 antibody is RG-7716, or wherein the soluble VEGF receptor fusion protein is aflibercept (EYLEA®), or wherein the aptamer is pegaptanib (MACUGEN®), or wherein the anti-VEGF DARPin® is abicipar pegol, or wherein the VEGFR tyrosine kinase inhibitor is selected from the group consisting of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474), 4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171), vatalanib (PTK787), semaxaminib (SU5416), and SUTENT® (sunitinib).

30. The use of claim29(i), wherein the second biological molecule is selected from the group consisting of IL-1β; IL-6; IL-6R; IL-13; IL-13R; PDGF; angiopoietin; angiopoietin 2; Tie2; S1P; integrins αvβ3, αvβ5, and α5β1; betacellulin; apelin/APJ; erythropoietin; complement factor D; TNFα; HtrA1; a VEGF receptor; ST-2 receptor; and a protein genetically linked to AMD risk; wherein optionally

the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, mbVEGFR, or sVEGFR; or

the protein genetically linked to AMD risk is selected from the group consisting of complement pathway components C2, factor B, factor H, CFHR3, C3b, C5, C5a, and C3a; HtrA1; ARMS2; TIMP3; HLA; IL-8; CX3CR1; TLR3; TLR4; CETP; LIPC, COL10A1; and TNFRSF10A.

31. The use of claim 29(i) or 30, wherein the compound that binds a second biological molecule is an antibody or antigen-binding fragment thereof; wherein optionally

the antigen-binding antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments.

32. The use of any one of claims 21-31, wherein (i) the antibody is to be administered intravitreally, ocularly, intraocularly, juxtasclerally, subtenonly, superchoroidally, topically, intravenously, intramuscularly, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intrathecally, intranasally, intravaginally, intrarectally, topically, intratumorally, intraperitoneally, peritoneally, intraventricularly, subcutaneously, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraorbitally, orally, transdermally, by inhalation, by injection, by eye drop, by implantation, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, in cremes, or in lipid compositions; wherein optionally (ii)

the antibody is to be administered intravitreally, ocularly, intraocularly, juxtasclerally, subtenonly, superchoroidally, or topically; wherein optionally (iii)

the antibody is to be administered intravitreally by injection, or

topically by eye drop or ointment.

33. The use of claim 32(i) or 32(ii) wherein the antibody is to be administered by a port delivery device.

34. The use of any one of claims 21-33, wherein the subject is a human.

35. A pharmaceutical composition comprising the antibody of any one of claims 1-15.

36. The pharmaceutical composition of claim 35, further comprising a polymer; wherein optionally

the polymer is a biodegradable polymer; or

wherein the pharmaceutical composition is formulated as a polymer solvent depot, a polymer implant, or a polymer micelle.

37. The pharmaceutical composition of claim 36, wherein the polymer is a polylactic acid polyglycolic acid (PLGA) copolymer; wherein optionally

the pharmaceutical composition is formulated as a PLGA rod.

38. The pharmaceutical composition of any one of claims 35-37, for use in the treatment of a disorder associated with pathological angiogenesis or a disorder associated with undesirable vascular permeability in a mammal.

39. The pharmaceutical composition of claim 38, wherein (i) the disorder associated with pathological angiogenesis is an ocular disorder or a cell proliferative disorder; wherein optionally (ii)

the disorder associated with pathological angiogenesis is an ocular disorder; wherein optionally (iii)

the ocular disorder is selected from the group consisting of AMD, macular degeneration, macular edema, DME (including focal, non-center DME and diffuse, center-involved DME), retinopathy, DR (including PDR, NPDR, and high-altitude DR), other ischemia-related retinopathies, ROP, RVO (including CRVO and BRVO forms), CNV (including myopic CNV), corneal neovascularization, a disease associated with corneal neovascularization, retinal neovascularization, a disease associated with retinal/choroidal neovascularization, pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, FEVR, Coats' disease, Norrie Disease, OPPG, subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, RP, hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, CME, vasculitis, papilloedema, retinitis, conjunctivitis (including infectious conjunctivitis and non-infectious (e.g., allergic) conjunctivitis), Leber congenital amaurosis, uveitis (including infectious and non-infectious uveitis), choroiditis, ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, and Sjögren's disease; and wherein optionally (iv)

the ocular disorder is AMD, DME, DR, or RVO.

40. The pharmaceutical composition of claim 39(iii) or 39(iv), wherein the ocular disorder is AMD; wherein optionally

the AMD is wet AMD.

41. The pharmaceutical composition of claim 39(i), wherein the disorder associated with pathological angiogenesis is a cell proliferative disorder; wherein optionally

the cell proliferative disorder is a cancer; and wherein optionally

wherein the cancer is selected from the group consisting of breast cancer, colorectal cancer, non-small cell lung cancer, non-Hodgkins lymphoma (NHL), renal cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, and multiple myeloma.

42. The pharmaceutical composition of claim 38, wherein the disorder associated with undesirable vascular permeability is selected from the group consisting of edema associated with brain tumors, ascites associated with malignancies, Meigs' syndrome, lung inflammation, nephrotic syndrome, pericardial effusion, pleural effusion, and permeability associated with cardiovascular diseases.

43. The pharmaceutical composition of any one of claims 35-42, (i) further comprising a second agent, wherein the second agent is selected from the group consisting of another antibody, a chemotherapeutic agent, a cytotoxic agent, an anti-angiogenic agent, an immunosuppressive agent, a prodrug, a cytokine, a cytokine antagonist, cytotoxic radiotherapy, a corticosteroid, an anti-emetic, a cancer vaccine, an analgesic, a growth-inhibitory agent, and a compound that binds to a second biological molecule; wherein optionally (ii)

the anti-angiogenic agent is a VEGF antagonist; wherein optionally (iii)

the VEGF antagonist is an anti-VEGF antibody, an anti-VEGF receptor antibody, a soluble VEGF receptor fusion protein, an aptamer, an anti-VEGF DARPin®, or a VEGFR tyrosine kinase inhibitor, wherein optionally the anti-VEGF antibody is ranibizumab (LUCENTIS®), RTH-258, or a bispecific anti-VEGF antibody, wherein optionally the bispecific anti-VEGF antibody is an anti-VEGF/anti-Ang2 antibody and wherein optionally the anti-VEGF/anti-Ang2 antibody is RG-7716, or wherein the soluble VEGF receptor fusion protein is aflibercept (EYLEA®), or wherein the aptamer is pegaptanib (MACUGEN®), or wherein the anti-VEGF DARPin® is abicipar pegol, or wherein the VEGFR tyrosine kinase inhibitor is selected from the group consisting of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474), 4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171), vatalanib (PTK787), semaxaminib (SU5416), and SUTENT® (sunitinib).

44. The pharmaceutical composition of claim 43(i), wherein the second biological molecule is selected from the group consisting of IL-1β; IL-6; IL-6R; IL-13; IL-13R; PDGF; angiopoietin; angiopoietin 2; Tie2; S1P; integrins αvβ3, αvβ5, and α5β1; betacellulin; apelin/APJ; erythropoietin; complement factor D; TNFα; HtrA1; a VEGF receptor; ST-2 receptor; and a protein genetically linked to AMD risk; wherein optionally

the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, mbVEGFR, or sVEGFR, or

the protein genetically linked to AMD risk is selected from the group consisting of complement pathway components C2, factor B, factor H, CFHR3, C3b, C5, C5a, and C3a; HtrA1; ARMS2; TIMP3; HLA; IL-8; CX3CR1; TLR3; TLR4; CETP; LIPC, COL10A1; and TNFRSF10A.

45. The pharmaceutical composition of claim 43(i) or 44, wherein the compound that binds a second biological molecule is an antibody or antigen-binding fragment thereof; wherein optionally

the antigen-binding antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments.

46. An antibody conjugate comprising (i) the antibody of any one of claims 1-15 and (ii) a hydrophilic polymer covalently attached to the antibody.

47. The antibody conjugate of claim 46, wherein (i) the hydrophilic polymer is a hyaluronic acid (HA) polymer or a polyethylene glycol (PEG) polymer; wherein optionally

(ii) the hydrophilic polymer is an HA polymer; and wherein optionally

(iii) the HA polymer has a molecular weight of about 1 megadalton (MDa) or lower.

48. An antibody conjugate comprising (i) an antibody that specifically binds VEGF and (ii) an HA polymer covalently attached to the antibody, wherein the HA polymer has a molecular weight of 1 MDa or lower.

49. The antibody conjugate of claim 47(iii) or 48, wherein (i) the HA polymer has a molecular weight between about 25 kDa and about 500 kDa; wherein optionally

(ii) the HA polymer has a molecular weight between about 100 kDa and about 250 kDa; and wherein optionally

(iii) the HA polymer has a molecular weight of about 200 kDa.

50. The antibody conjugate of any one of claims 46-49, wherein (i) the HA polymer is not cross-linked; wherein optionally

(ii) the antibody is an antibody fragment that binds VEGF, wherein optionally

(iii) the antibody fragment is selected from the group consisting of Fab, Fab-C, Fab', Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments, wherein optionally

(iv) the antibody fragment is an Fab, Fab-C, or Fab'; wherein optionally

(v) the antibody conjugate has a hydrodynamic radius between about 10 nm and about 60 nm, wherein optionally

(vi) the antibody conjugate has a hydrodynamic radius between about 25 nm and about 35 nm, wherein optionally

(vii) the hydrodynamic radius is about 28 nm; and wherein optionally

(viii) the antibody conjugate has an ocular half-life that is increased relative to a reference antibody that is not covalently attached to the hydrophilic polymer, wherein optionally

(ix) the ocular half-life is increased at least about 2-fold relative to the reference antibody, and wherein optionally

(x) the ocular half-life is increased at least about 4-fold relative to the reference antibody.

51. The antibody conjugate of any one of claims 50(viii)-50(x), wherein the ocular half-life is a vitreal half-life; wherein optionally

the reference antibody is identical to the antibody of the antibody conjugate.

52. The antibody conjugate of claim 46, wherein (i) the antibody is covalently attached to the polymer by a reversible prodrug linker; wherein optionally

(ii) the polymer is a hydrogel; wherein optionally

(iii) the hydrogel is a PEG-based hydrogel.

53. The antibody conjugate of claim 52(ii) or 52(iii), wherein the hydrogel is in the shape of a microparticulate bead.

54. A fusion protein comprising the antibody of any one of claims 1-15 covalently attached to an HA binding domain.

55. The fusion protein of claim 54, wherein (i) the HA binding domain is covalently attached to the heavy chain or the light chain of the antibody; wherein optionally

(ii) the HA binding domain is covalently attached to the C-terminus of the heavy chain or to the C-terminus of the light chain, wherein optionally

(iii) the fusion protein further comprises a linker, the linker being positioned between the antibody and the HA binding domain, wherein optionally

(iv) the linker comprises the amino acid sequence of GGGGS (SEQ ID NO: 61), wherein optionally

(v) the linker consists of the amino acid sequence of GGGGS (SEQ ID NO: 61); wherein optionally

(vi) the antibody is an antibody fragment that binds VEGF, wherein optionally

(vii) the antibody fragment is selected from the group consisting of Fab, Fab-C, Fab', Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments, wherein optionally

(viii) the antibody fragment is an Fab, wherein optionally

(ix) the HA binding domain is covalently attached to the C-terminus of the CH1 domain of the Fab, or

the CL domain of the Fab; wherein optionally

(x) the HA binding domain is selected from the group consisting of a link module, a G1 domain, and a lysine-rich oligopeptide, wherein optionally

(xi) the HA binding domain is a link module, wherein optionally

(xii) the link module is selected from the group consisting of tumor necrosis factor-stimulated gene 6 (TSG6), CD44, lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1), hyaluronan and proteoglycan link protein (HAPLN) 1, HAPLN2, HAPLN3, HAPLN4, aggrecan, brevican, neurocan, phosphacan, versican, CAB61358, KIA0527, stabilin-1, and stabilin-2 link modules, wherein optionally

(xiii) the link module is a TSG6 link module, wherein optionally

(xiv) the TSG6 link module is a human TSG6 link module or a rabbit TSG6 link module, wherein optionally

(xv) the TSG link module is a human TSG6 link module; wherein optionally

(xvi) the human TSG6 link module comprises amino acid residues 36-128 of human TSG6; and wherein optionally

(xvii) the fusion protein further comprises at least one additional HA binding domain, wherein optionally

(xviii) the at least one additional HA binding domain is covalently attached to the heavy chain or the light chain of the antibody.

56. The fusion protein of claim 55(xvii) or 55(xviii), wherein (i) the at least one additional HA binding protein is linked to the antibody by a linker; wherein optionally

(ii) the linker comprises the amino acid sequence of GGGGS (SEQ ID NO: 61), wherein optionally

(iii) the linker consists of the amino acid sequence of GGGGS (SEQ ID NO: 61); wherein optionally

(iv) a first HA binding domain is covalently attached to the heavy chain and a second HA binding domain is covalently attached to the light chain, wherein optionally

(v) the first HA binding domain is linked to the C-terminus of the heavy chain and the second HA binding domain is linked to the C-terminus of the light chain; wherein optionally

(vi) the at least one additional HA binding protein is selected from the group consisting of a link module, a G1 domain, and a lysine-rich oligopeptide, wherein optionally

(vii) the at least one additional HA binding protein is a link module, wherein optionally

(viii) the link module is a TSG6 link module, wherein optionally

(ix) the TSG6 link module is a human TSG6 link module or a rabbit TSG6 link module, wherein optionally

(x) the TSG6 link module is a human TSG6 link module.

57. The fusion protein of claim 56(ix) or 56(x), wherein the human TSG6 link module comprises amino acid residues 36-128 of human TSG6.

58. The fusion protein of any one of claims 54-57, wherein (i) the fusion protein specifically binds to VEGF and HA; wherein optionally

(ii) the fusion protein binds HA with a Kd of about 2 μM or lower, wherein optionally

(iii) the fusion protein binds HA with a Kd between about 1 nM and about 500 nM, wherein optionally

(iv) the fusion protein binds HA with a Kd between about 1 nM and about 50 nM, wherein optionally

(v) the fusion protein binds HA with a Kd of about 10 nM; and wherein optionally

(vi) the fusion protein has an ocular half-life that is increased relative to a reference antibody that is not covalently attached to a HA binding domain, wherein optionally

(vii) the ocular half-life is increased at least about 2-fold relative to the reference antibody, and wherein optionally

(viii) the ocular half-life is increased at least about 4-fold relative to the reference antibody.

59. The fusion protein of any one of claims 58(vi)-58(viii), wherein the ocular half-life is a vitreal half-life; wherein optionally

the reference antibody is identical to the antibody of the fusion protein.

60. Use of the antibody conjugate of any one of claims 46-53 in the manufacture of a medicament for reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis .

61. Use of the antibody conjugate of any one of claims 46-53 in the manufacture of a medicament for treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

62. Use of the fusion protein of any one of claims 54-59 in the manufacture of a medicament for reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

63. Use of the fusion protein of any one of claims 54-59 in the manufacture of a medicament for treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

64. The use of any one of claims 60-63, wherein (i) the disorder associated with pathological angiogenesis is an ocular disorder; wherein optionally

(ii) the ocular disorder is selected from the group consisting of AMD, macular degeneration, macular edema, DME (including focal, non-center DME and diffuse, center-involved DME), retinopathy, DR (including PDR, NPDR, and high-altitude DR), other ischemia-related retinopathies, ROP, RVO (including CRVO and BRVO forms), CNV (including myopic CNV), corneal neovascularization, a disease associated with corneal neovascularization, retinal neovascularization, a disease associated with retinal/choroidal neovascularization, pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, FEVR, Coats' disease, Norrie Disease, OPPG, subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, RP, hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, CME, vasculitis, papilloedema, retinitis, conjunctivitis (including infectious conjunctivitis and non-infectious (e.g., allergic) conjunctivitis), Leber congenital amaurosis, uveitis (including infectious and non-infectious uveitis), choroiditis, ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, and Sjögren's disease; wherein optionally

(iii) the ocular disorder is AMD, DME, DR, or RVO; wherein optionally

(iv) the ocular disorder is AMD.

65. The use of any one of claims 64(ii)-64(iv), wherein the AMD is wet AMD.

66. The use of any one of claims 60-65, wherein (i) the medicament is to be administered with a second agent, wherein the second agent is selected from the group consisting of another antibody, an anti-angiogenic agent, a cytokine, a cytokine antagonist, a corticosteroid, an analgesic, and a compound that binds to a second biological molecule; wherein optionally

(ii) the anti-angiogenic agent is a VEGF antagonist; wherein optionally

(iii) the VEGF antagonist is an anti-VEGF antibody, an anti-VEGF receptor antibody, a soluble VEGF receptor fusion protein, an aptamer, an anti-VEGF DARPin®, or a VEGFR tyrosine kinase inhibitor; wherein optionally

(iv) the anti-VEGF antibody is ranibizumab (LUCENTIS®), RTH-258, or a bispecific anti-VEGF antibody; wherein optionally

(v) the bispecific anti-VEGF antibody is an anti-VEGF/anti-Ang2 antibody; and wherein optionally

(vi) the anti-VEGF/anti-Ang2 antibody is RG-7716.

67. The use of claim 66(iii), wherein the soluble VEGF receptor fusion protein is aflibercept (EYLEA®), or

wherein the aptamer is pegaptanib (MACUGEN®), or

wherein the anti-VEGF DARPin® is abicipar pegol, or

wherein the VEGFR tyrosine kinase inhibitor is selected from the group consisting of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474), 4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171), vatalanib (PTK787), semaxaminib (SU5416), and SUTENT® (sunitinib).

68. The method of claim 66(i), wherein the second biological molecule is selected from the group consisting of IL-1β; IL-6; IL-6R; IL-13; IL-13R; PDGF; angiopoietin; angiopoietin 2; Tie2; S1P; integrins αvβ3, αvβ5, and α5β1; betacellulin; apelin/APJ; erythropoietin; complement factor D; TNFα; HtrA1; a VEGF receptor; ST-2 receptor; and a protein genetically linked to AMD risk; wherein optionally

the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, mbVEGFR, or sVEGFR, or

the protein genetically linked to AMD risk is selected from the group consisting of complement pathway components C2, factor B, factor H, CFHR3, C3b, C5, C5a, and C3a; HtrA1; ARMS2; TIMP3; HLA; IL-8; CX3CR1; TLR3; TLR4; CETP; LIPC, COL10A1; and TNFRSF10A.

69. The use of of claim 66(i) or 68, wherein the compound that binds a second biological molecule is an antibody or antigen-binding fragment thereof; wherein optionally

the antigen-binding antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments.

70. The use of any one of claims 60, 61, and 64-69, wherein (i) the medicament is to be administered intravitreally, ocularly, intraocularly, juxtasclerally, subtenonly, superchoroidally, topically, intravenously, intramuscularly, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intrathecally, intranasally, intravaginally, intrarectally, topically, intratumorally, intraperitoneally, peritoneally, intraventricularly, subcutaneously, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraorbitally, orally, transdermally, by inhalation, by injection, by eye drop, by implantation, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, in cremes, or in lipid compositions; wherein optionally

(ii) the medicament is to be administered intravitreally, ocularly, intraocularly, juxtasclerally, subtenonly, superchoroidally, or topically; wherein optionally

(iii) the medicament is to be administered intravitreally by injection, or

topically by eye drop or ointment.

71. The use of claim 70(i) or 70(ii), wherein the medicament is to be administered by a port delivery device.

72. The use of any one of claims 62-71, wherein (i) the medicament is to be administered intravitreally, ocularly, intraocularly, juxtasclerally, subtenonly, superchoroidally, topically, intravenously, intramuscularly, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intrathecally, intranasally, intravaginally, intrarectally, topically, intratumorally, intraperitoneally, peritoneally, intraventricularly, subcutaneously, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraorbitally, orally, transdermally, by inhalation, by injection, by eye drop, by implantation, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, in cremes, or in lipid compositions; wherein optionally

(ii) the medicament is to be administered intravitreally, ocularly, intraocularly, juxtasclerally, subtenonly, superchoroidally, or topically; and wherein optionally

(iii) the medicament is to be administered intravitreally by injection, or

topically by eye drop or ointment.

73. The use of claim 72(i) or 72(ii), wherein the medicament is to be administered by a port delivery device.

74. The use of any one of claims 60-73, wherein the subject is a human.

75. A pharmaceutical composition comprising the antibody conjugate of any one of claims 4653.

76. A pharmaceutical composition comprising the fusion protein of any one of claims 54-59.

77. The pharmaceutical composition of claim 75 or 76, for use in the treatment of a disorder associated with pathological angiogenesis in a mammal.

78. The pharmaceutical composition of claim 77, wherein (i) the disorder associated with pathological angiogenesis is an ocular disorder; wherein optionally

(ii) the ocular disorder is selected from the group consisting of AMD, macular degeneration, macular edema, DME (including focal, non-center DME and diffuse, center-involved DME), retinopathy, DR (including PDR, NPDR, and high-altitude DR), other ischemia-related retinopathies, ROP, RVO (including CRVO and BRVO forms), CNV (including myopic CNV), corneal neovascularization, a disease associated with corneal neovascularization, retinal neovascularization, a disease associated with retinal/choroidal neovascularization, pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, FEVR, Coats' disease, Norrie Disease, OPPG, subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, RP, hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, CME, vasculitis, papilloedema, retinitis, conjunctivitis (including infectious conjunctivitis and non-infectious (e.g., allergic) conjunctivitis), Leber congenital amaurosis, uveitis (including infectious and non-infectious uveitis), choroiditis, ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, and Sjögren's disease; and wherein optionally

(iii) the ocular disorder is AMD, DME, DR, or RVO.

79. The pharmaceutical composition of claim 78(ii) or 78(iii), wherein the ocular disorder is AMD.

80. The pharmaceutical composition of any one of claims 78(ii), 78(iii) and 79, wherein the AMD is wet AMD.

81. The pharmaceutical composition of any one of claims 75-80, further comprising a second agent, wherein (i) the second agent is selected from the group consisting of another antibody, an anti-angiogenic agent, a cytokine, a cytokine antagonist, a corticosteroid, an analgesic, and a compound that binds to a second biological molecule; wherein optionally

(ii) the anti-angiogenic agent is a VEGF antagonist; wherein optionally

(iii) the VEGF antagonist is an anti-VEGF antibody, an anti-VEGF receptor antibody, a soluble VEGF receptor fusion protein, an aptamer, an anti-VEGF DARPin®, or a VEGFR tyrosine kinase inhibitor; wherein optionally

(iv) the anti-VEGF antibody is ranibizumab (LUCENTIS®), RTH-258, or a bispecific anti-VEGF antibody; wherein optionally

(v) the bispecific anti-VEGF antibody is an anti-VEGF/anti-Ang2 antibody; and wherein optionally

(vi) the anti-VEGF/anti-Ang2 antibody is RG-7716.

82. The pharmaceutical composition of claim 81(iii), wherein the soluble VEGF receptor fusion protein is aflibercept (EYLEA®), or

wherein the aptamer is pegaptanib (MACUGEN®), or

wherein the anti-VEGF DARPin® is abicipar pegol, or

wherein the VEGFR tyrosine kinase inhibitor is selected from the group consisting of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474), 4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171), vatalanib (PTK787), semaxaminib (SU5416), and SUTENT® (sunitinib).

83. The pharmaceutical composition of claim 81(i), wherein the second biological molecule is selected from the group consisting of IL-1β; IL-6; IL-6R; IL-13; IL-13R; PDGF; angiopoietin; angiopoietin 2; Tie2; S1P; integrins αvβ3, αvβ5, and α5β1; betacellulin; apelin/APJ; erythropoietin; complement factor D; TNFα; HtrA1; a VEGF receptor; ST-2 receptor; and a protein genetically linked to AMD risk; wherein optionally

(ii) the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, mbVEGFR, or sVEGFR, or

(iii) the protein genetically linked to AMD risk is selected from the group consisting of complement pathway components C2, factor B, factor H, CFHR3, C3b, C5, C5a, and C3a; HtrA1; ARMS2; TIMP3; HLA; IL-8; CX3CR1; TLR3; TLR4; CETP; LIPC, COL10A1; and TNFRSF10A.

84. The pharmaceutical composition of any one of claims 81(i)and 83(ii)-83(iii), wherein the compound that binds a second biological molecule is an antibody or antigen-binding fragment thereof; wherein optionally

the antigen-binding antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments.

85. A method of identifying an amino acid residue alteration that confers enhanced binding of an antibody to a target molecule, the method comprising:

(a) providing a display library comprising nucleic acids encoding candidate antibody variants, wherein each candidate antibody variant comprises an amino acid residue alteration in the VH or the VL compared to a reference antibody, and wherein amino acid residue alterations at every position of the VH or VL are present in the display library;

(b) sorting the display library based on binding of the candidate antibody variants to the target molecule to form a sorted library, wherein the sorted library comprises candidate antibody variants with enhanced binding to the target molecule compared to the reference antibody; and

(c) comparing the frequency at which each amino acid residue alteration is present in the display library and in the sorted library as determined by massively parallel sequencing, thereby determining whether each amino acid residue alteration is enriched in the sorted library compared to the display library,

whereby the amino acid residue alteration is identified as conferring enhanced binding to the target molecule if it is enriched in the sorted library compared to the display library.

86. A method of identifying an amino acid residue alteration that confers enhanced stability to an antibody, the method comprising:

(a) providing a display library comprising nucleic acids encoding candidate antibody variants, wherein each candidate antibody variant comprises an amino acid residue alteration in the VH or the VL compared to a reference antibody, and wherein amino acid residue alterations at every position of the VH or VL are present in the display library;

(b) sorting the display library based on binding of the candidate antibody variants to the target molecule to form a sorted library, wherein the sorted library comprises candidate antibody variants with enhanced stability compared to the reference antibody; and

(c) comparing the frequency at which each amino acid residue alteration is present in the display library and in the sorted library as determined by massively parallel sequencing, thereby determining

whether each amino acid residue alteration is enriched in the sorted library compared to the display library,

whereby the amino acid residue alteration is identified as conferring enhanced stability to the antibody if it is enriched in the sorted library compared to the display library.

87. The method of claim 85 or 86, (i) further comprising determining the frequency at which each amino acid alteration is present in the display library and the sorted library by massively parallel sequencing following step (b); wherein optionally

(ii) the amino acid residue alteration is enriched at least 2-fold in the sorted library compared to the display library; wherein optionally

(iii) the display library is selected from the group consisting of a phage display library, a bacterial display library, a yeast display library, a mammalian display library, a ribosome display library, and an mRNA display library, wherein optionally

(iv) the display library is a phage display library; wherein optionally

(v) the amino acid residue alteration is encoded by a degenerate codon set, wherein optionally

(vi) the degenerate codon set is an *NNK* or an *NNS* codon set, wherein *N* is A, C, G, or T; *K* is G or T; and *S* is C or G, wherein optionally

(vii) the degenerate codon set is an *NNK* codon set; wherein optionally

(viii) the sorting of step (b) comprises contacting the display library with an immobilized target molecule or epitope; wherein optionally

(ix) the sorting of step (b) comprises contacting the display library with a soluble target molecule or epitope; wherein optionally

(x) the display library comprises at least $1x10^6$ candidate antibody variants, wherein optionally

(xi) the display library comprises at least $1x10^8$ antibody variants, wherein optionally

(xii) the display library comprises at least $1x10^9$ antibody variants; wherein optionally

(xiii) the massively parallel sequencing comprises deep sequencing, ultra-deep sequencing, and/or next-generation sequencing; wherein optionally

(xiv) the antibody is a monoclonal antibody; and wherein optionally

(xv) the antibody is an IgG antibody, or

(xvi) an antibody fragment.

88. The method of claim 87(xvi), wherein the antibody fragment is selected from the group consisting of Fab, scFv, Fv, Fab', Fab-C, Fab'-SH, $F(ab')_2$, and diabody; wherein optionally

the antibody fragment is an Fab.

89. The method of any one of claims 85-88, wherein the method further comprises generating an antibody that comprises an amino acid residue alteration identified by the steps of the method.

90. The antibody of any one of claims 1-15 for use in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

91. The antibody of any one of claims 1-15 for use in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

92. The antibody of any one of claims 1-15 for use in a method of inhibiting vascular permeability in a subject suffering from a disorder associated with undesirable vascular permeability.

93. A composition comprising the antibody of any one of claims 1-15 for use in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

94. A composition comprising the antibody of any one of claims 1-15 for use in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

95. A composition comprising the antibody of any one of claims 1-15 for use in a method of inhibiting vascular permeability in a subject suffering from a disorder associated with undesirable vascular permeability.

96. The antibody conjugate of any one of claims 46-53 for use in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

97. The antibody conjugate of any one of claims 46-53 for use in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

98. A composition comprising the antibody conjugate of any one of claims 46-53 for use in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

99. A composition comprising the antibody conjugate of any one of claims 46-53 for use in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

100. The fusion protein of any one of claims 54-59 for use in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

101. The fusion protein of any one of claims 54-59 for use in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.

102. A composition comprising the fusion protein of any one of claims 54-59 for use in a method of reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

103. A composition comprising the fusion protein of any one of claims 54-59 for use in a method of treating a disorder associated with pathological angiogenesis in a subject in need of such treatment.