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

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

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

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

In some examples of any of the preceding aspects, the antibody is a monospecific antibody. In other examples of any of the preceding aspects, the antibody is a multispecific antibody. In some examples, the multispecific antibody is a bispecific antibody. In some examples, 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 examples, the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, membrane-bound VEGF-receptor (mbVEGFR), or soluble VEGF receptor (sVEGFR). In some examples, 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.

In some examples of any of the preceding aspects, the antibody is a cysteine engineered antibody. In some examples, the cysteine engineered antibody comprises a cysteine mutation in the heavy chain selected from the group consisting of HC-A118C, HC-A140C, and HC-L174C (EU numbering), or a cysteine mutation in the light chain selected from the group consisting of LC-K149C and LC-V205C (Kabat numbering). In some examples, the cysteine mutation in the heavy chain is HC-A118C (EU numbering). In some examples, the cysteine mutation in the heavy chain is HC-A140C (EU numbering). In some examples, the cysteine mutation in the heavy chain is HC-L174C (EU numbering). In some examples, the cysteine mutation in the light chain is LC-K149C (Kabat numbering). In some examples, the cysteine mutation in the light chain is LC-V205C (Kabat numbering). In some examples, the HA polymer is covalently attached to the antibody at the cysteine mutation.

In another aspect, the present disclosure refers to 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 PDI of 1.05, and 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.

In yet another aspect, the present disclosure refers to 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 PDI of 1.05, and wherein the antibody comprises (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 48 or SEQ ID NO: 49 and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 50.

In one example, any of the preceding antibody conjugates can be used as a medicament.

In another example, any of the preceding antibody conjugates can be used in the manufacture of a medicament for treating an ocular disorder in a subject. In one aspect, the present disclosure refers to use of any of the preceding antibody conjugates in the manufacture of a medicament for (i) treating an ocular disorder in a subject or (ii) reducing or inhibiting angiogenesis in a subject having an ocular disorder, wherein the ocular disorder is AMD, DME, DR, or RVO.

In another example, any of the preceding antibody conjugates can be used in reducing or inhibiting angiogenesis in a subject having an ocular disorder.

In another example any of the preceding antibody conjugates can be used in treating an ocular disorder in a subject.

In one aspect, the present disclosure refers to any of the preceding antibody conjugates for use in (i) treating an ocular disorder in a subject or (ii) reducing or inhibiting angiogenesis in a subject having an ocular disorder, wherein the ocular disorder is AMD, DME, DR, or RVO.

In some examples of any of the preceding aspects, 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 examples, the ocular disorder is AMD, DME, DR, or RVO. In some examples, the ocular disorder is AMD. In some examples, the AMD is wet AMD. In some examples, the ocular disorder is DME.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising any of the antibody conjugates described herein and a pharmaceutically acceptable carrier, excipient, or diluent. In one example, the present disclosure refers to a pharmaceutical composition comprising any of the antibody conjugates described herein for use in (i) treating an ocular disorder in a subject or (ii) reducing or inhibiting angiogenesis in a subject having an ocular disorder. In some examples, the pharmaceutical composition further comprises a second agent, wherein the second agent is selected from the group consisting of an 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 examples, the anti-angiogenic agent is a VEGF antagonist. In some examples, 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 examples, the anti-VEGF antibody is ranibizumab (LUCENTIS®), RTH-258, or a bispecific anti-VEGF antibody. In some examples, the bispecific anti-VEGF antibody is an anti-VEGF/anti-Ang2 antibody. In some examples, the anti-VEGF/anti-Ang2 antibody is RG-7716. In some examples, the soluble VEGF receptor fusion protein is aflibercept (EYLEA®). In some examples, the aptamer is pegaptanib (MACUGEN®). In some examples, the anti-VEGF DARPin® is abicipar pegol. In some examples, 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 examples, the second biological molecule is selected from the group consisting of IL-1β; IL-6; IL-6R; IL-13; IL-13R; PDGF; angiopoietin;

CLAIMS

What is claimed is:

1. An antibody conjugate comprising (i) an antibody that specifically binds to vascular endothelial growth factor (VEGF) and (ii) a hyaluronic acid (HA) polymer covalently attached to the antibody, wherein the HA polymer has a polydispersity index (PDI) between 1.0 and 1.1, wherein the HA polymer has a molecular weight of 1 megadalton (MDa) or lower, and wherein 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).

2. The antibody conjugate of claim 1, wherein the HA polymer has a PDI between 1.0 and 1.07.

3. The antibody conjugate of claim 1 or 2, wherein the HA polymer has a PDI of 1.05.

4. The antibody conjugate of any one of claims 1-3, wherein the HA polymer has a molecular weight between 25 kDa and 500 kDa, optionally wherein the HA polymer has a molecular weight between 100 kDa and 250 kDa, further optionally wherein the HA polymer has a molecular weight between 150 kDa and 200 kDa.

5. The antibody conjugate of any one of claims 1-4, wherein the HA polymer is a linear HA polymer.

6. The antibody conjugate of any one of claims 1-5, wherein the antibody conjugate has a hydrodynamic radius between 10 nm and 60 nm, optionally wherein the antibody conjugate has a hydrodynamic radius between 25 nm and 35 nm, further optionally wherein the hydrodynamic radius is 20 nm to 30 nm.

7. The antibody conjugate of any one of claims 1-6, wherein the antibody conjugate has an ocular half-life that is increased relative to a reference antibody that is not covalently attached to the HA polymer, optionally wherein the ocular half-life is increased at least 2-fold or at least 4-fold relative to the reference antibody, further optionally wherein the ocular half-life is a vitreal half-life, further optionally wherein the reference antibody is identical to the antibody of the antibody conjugate.

8. The antibody conjugate of any one of claims 1-7, 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).

9. The antibody conjugate of any one of claims 1-8, wherein 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).

10. The antibody conjugate of any one of claims 1-9, 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 and (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.

11. The antibody conjugate of claim 10, wherein the VH domain comprises the amino acid sequence of SEQ ID NO: 11.

12. The antibody conjugate of claim 10 or 11, wherein the VL domain comprises the amino acid sequence of SEQ ID NO: 12.

13. The antibody conjugate of claim 11 or 12, wherein the antibody comprises (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 48 or SEQ ID NO: 49 and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 50.

14. The antibody conjugate of any one of claims 1-13, wherein:

(i) the antibody is capable of inhibiting the binding of VEGF to a VEGF receptor, optionally wherein the VEGF receptor is VEGF receptor 1 (Flt-1) or VEGF receptor 2 (KDR);

(ii) the antibody binds human VEGF (hVEGF) with a Kd of 2 nM or lower, optionally wherein the antibody binds hVEGF with a Kd between 75 pM and 2 nM, further optionally wherein the antibody binds hVEGF with a Kd between 75 pM and 600 pM, further optionally wherein the antibody binds hVEGF with a Kd between 75 pM and 500 pM, further optionally wherein the antibody binds hVEGF with a Kd of 60 pM;

(iii) the antibody has a melting temperature (Tm) of greater than 83.5°C, optionally wherein the antibody has a Tm of 85°C to 91°C, further optionally wherein the antibody has a Tm of 89°C; and/or

(iv) the antibody has an isoelectric point (pI) of lower than 8, optionally wherein the antibody has a pI of from 5 to 7, further optionally wherein the antibody has a pI of from 5 to 6.

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

16. The antibody conjugate of any one of claims 1-14, wherein the antibody is an antigen-binding antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments.

17. The antibody conjugate of claim 16, wherein the antibody fragment is an Fab or Fab-C.

18. The antibody conjugate of any one of claims 1-14, wherein the antibody is a monospecific antibody.

19. The antibody conjugate of any one of claims 1-14, wherein the antibody is a multispecific antibody, optionally wherein the multispecific antibody is a bispecific antibody, further optionally wherein the bispecific antibody binds VEGF and a second biological molecule 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, optionally wherein the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, mbVEGFR, or sVEGFR; ST-2 receptor; and a protein genetically linked to AMD risk, optionally wherein 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.

20. The antibody conjugate of any one of claims 1-14, wherein the antibody is a cysteine engineered antibody, optionally wherein the cysteine engineered antibody comprises a cysteine mutation in the heavy chain selected from the group consisting of HC-A118C, HC-A140C, and HC-L174C (EU numbering), or a cysteine mutation in the light chain selected from the group consisting of LC-K149C and LC-V205C

(Kabat numbering), further optionally wherein the HA polymer is covalently attached to the antibody at the cysteine mutation.

21. 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 PDI of 1.05, and 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.

22. 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 PDI of 1.05, and wherein the antibody comprises (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 48 or SEQ ID NO: 49 and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 50.

23. The antibody conjugate of any one of claims 1-22 for use in (i) treating an ocular disorder in a subject or (ii) reducing or inhibiting angiogenesis in a subject having an ocular disorder, wherein the ocular disorder is AMD, DME, DR, or RVO.

24. A pharmaceutical composition comprising the antibody conjugate of any one of claims 1-22 and a pharmaceutically acceptable carrier, excipient, or diluent.

25. The pharmaceutical composition of claim 24, further comprising a second agent, wherein the second agent is selected from the group consisting of an antibody, an anti-angiogenic agent, a cytokine, a cytokine antagonist, a corticosteroid, an analgesic, and a compound that binds to a second biological molecule, optionally wherein:

(i) the anti-angiogenic agent is a VEGF antagonist, optionally wherein 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, further optionally wherein the anti-VEGF antibody is ranibizumab (LUCENTIS®), RTH-258, or a bispecific anti-VEGF antibody, further optionally wherein the bispecific anti-VEGF antibody is an anti-VEGF/anti-Ang2 antibody, further optionally wherein the anti-VEGF/anti-Ang2 antibody is RG-7716, further optionally wherein the soluble VEGF receptor fusion protein is aflibercept (EYLEA®), further optionally wherein the aptamer is pegaptanib (MACUGEN®), further optionally wherein the anti-VEGF DARPin® is abicipar pegol, further optionally 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); or

(ii) 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, optionally wherein the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, mbVEGFR, or sVEGFR; ST-2 receptor; and a protein genetically linked to AMD risk, optionally wherein 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, further optionally the compound that binds a second biological molecule is an antibody or antigen-binding fragment thereof, further optionally wherein the antigen-binding antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments.

26. Use of the antibody conjugate of any one of claims 1-22 in the manufacture of a medicament for (i) treating an ocular disorder in a subject or (ii) reducing or inhibiting angiogenesis in a subject having an ocular disorder, wherein the ocular disorder is AMD, DME, DR, or RVO.