

[0006] In 1996, in the course of structure-function studies aiming to identify VEGF binding elements in VEGFR1, we found that deletion of Ig-like domain (D) 2, but not of other Ds, abolished VEGF or PlGF binding ²⁷. Replacing D2 of VEGFR3 with VEGFR1 D2 conferred on VEGFR3 the ligand specificity of VEGFR1²⁷. Subsequent studies documented the interaction between D2 and VEGF (or PlGF) by X-ray crystallography ²⁸⁻³⁰. However, D3 was important for optimal VEGF binding^{27 28}. These initial studies led to the design of a construct comprising the first three Ig-like Ds of VEGFR1, fused to an Fc-IgG (Flt-1-3-IgG) ²⁷. Flt-1-3-IgG showed a potent ability to neutralize VEGF *in vitro* and in several *in vivo* models of physiological and pathological angiogenesis ^{31-34 35 36}. However, the half-life of this molecule following systemic administration was relatively short due to the presence of clusters of basic residues in D3, which resulted in binding to heparan sulfate proteoglycans (HSPG) and sequestration in various tissues.

[0007] In 2002 Holash et al ²¹ described an IgG fusion construct comprising of VEGFR1 D2 and VEGFR2 D3, which has much lower heparin-affinity than VEGFR1 D3. This molecule, known today as aflibercept, ziv-aflibercept or EYLEA, was reported to have a significantly longer systemic half-life than Flt-(1-3-IgG) ²¹. These PK characteristics, combined with high binding affinity for VEGF and the ability to bind PlGF and VEGF-B, led to the prediction that aflibercept would be a more effective anti-tumor agent than other VEGF inhibitors ^{21 37}. However, aflibercept has gained FDA approval only for 2nd line treatment of colorectal cancer, while bevacizumab and the anti-VEGFR2 antibody ramucirumab received several FDA approvals in multiple cancer types ^{3 17}, suggesting that the above mentioned characteristics did not provide a therapeutic advantage. Clearly, aflibercept has had its major clinical impact as an intravitreal treatment for ocular vascular disorders.

SUMMARY

[0008] The present disclosure provides compositions and methods for inhibiting angiogenesis and for treating VEGF-associated conditions, such as ocular disease, including but not limited to, age-related macular degeneration, proliferative diabetic retinopathy, retinal vein occlusion, choroidal neovascularization secondary to myopia, retinopathy of prematurity, diabetic macular edema, polypoidal choroidal vasculopathy,

comprising administering an anti-VEGF agent that inhibits the activity of VEGF and, at the same time, has strong heparin-binding characteristics, thereby providing superior pharmacokinetics, namely having a longer half-life of the therapeutic agent following intravitreal administration.

[0009] The present disclosure provides compositions and methods for treating a VEGF-related ophthalmic disorder in a subject in need comprising, administering intravitreally to the subject a first therapeutically effective amount of an anti-VEGF agent, and administering intravitreally to the subject a second therapeutically effective amount of the anti-VEGF agent within 10 to 30 weeks of the earlier administration. In examples, the anti-VEGF agent comprises a VEGF binding portion operatively linked to a Fc-IgG, wherein the VEGF binding portion comprises at least one VEGF binding domain that is an IgG-like domain 2 of VEGFR-1.

[0010] In examples, the second therapeutically effective amount of the anti-VEGF agent is administered intravitreally within 16 to 24 weeks of the earlier administration. In examples, the method comprises subsequent administrations of the therapeutically effective amount of the anti-VEGF agent administered intravitreally within 10 to 30 weeks of a prior administration for a period of at least one year.

[0011] In examples, the therapeutically effective amount of the anti-VEGF agent is about 1 to 10 mg. In examples, the therapeutically effective amount of the anti-VEGF agent is about 3 to 6 mg. In examples, the first, second and subsequent therapeutically effective amounts are the same. In examples, the first, second and subsequent therapeutically effective amounts are different.

[0012] The present disclosure provides an anti-VEGF agent, wherein the anti-VEGF agent is an Fc-IgG construct fusing domains with VEGF binding characteristics and domains that bind heparin proteoglycans. The present disclosure provides an anti-VEGF agent, wherein the anti-VEGF agent is an Fc-IgG construct having the ability to bind heparin and contains one or more domains with VEGF binding characteristics. The present disclosure provides an anti-VEGF agent, wherein the anti-VEGF agent is a fusion protein with improved efficacy for binding

to VEGF and heparin. The present disclosure provides an anti-VEGF agent, wherein the anti-VEGF agent is a fusion protein with very low endotoxin levels.

[0013] The present disclosure provides an anti-VEGF agent, wherein the anti-VEGF agent is an IgG chimeric protein comprising elements of VEGF receptors. The present disclosure provides an IgG chimeric protein, wherein the IgG chimeric protein comprises one or more fragments of the seven immunoglobulin (Ig)-like domains in the extracellular portion of VEGF tyrosine kinase receptors. The present disclosure provides an IgG chimeric protein, wherein the IgG chimeric protein comprises one or more extracellular domain fragments of VEGFR-1 fused with Fc-IgG. The present disclosure provides an IgG chimeric protein comprising at least one VEGF binding domain VEGFR-1 domain 2 and at least one additional VEGFR-1 domain 1 or 3, and not including domain 4. The present disclosure provides an IgG chimeric protein, wherein the IgG chimeric protein comprises one or more extracellular domain fragments of VEGFR-2 fused with Fc-IgG. The present disclosure provides an IgG chimeric protein, wherein the IgG chimeric protein comprises one or more extracellular domain fragments of VEGFR-1 and VEGFR-2 fused with Fc-IgG.

[0014] The present disclosure provides an anti-VEGF agent comprising a VEGF binding portion operatively linked to a Fc-IgG, wherein the VEGF binding portion comprises at least one VEGF binding domain that is an IgG-like domain 2 of VEGFR-1, and wherein the anti-VEGF agent has a VEGF-stimulated mitogenesis-inhibiting ability greater than aflibercept. In examples, the present disclosure provides that the anti-VEGF agent has a vitreous binding ability greater than aflibercept. In examples, the present disclosure provides that the anti-VEGF agent has a vitreous bound VEGF-stimulated endothelial cell proliferation-inhibiting ability greater than aflibercept. In examples, the present disclosure provides that the agent has an increased half-life *in vivo* compared to aflibercept.

[0015] In examples, the present disclosure provides that the VEGF binding portion consists essentially of IgG-like domains 1, 2, and 3 of VEGFR-1 (V₁₋₂₋₃).

[0016] In examples, the present disclosure provides that the VEGF binding portion consists essentially of IgG-like domains 2 and 3 of VEGFR-1 (V₂₋₃).

[0017] In examples, the present disclosure provides that the VEGF binding portion consists essentially of IgG-like domains 1, 2, 3 and 3 of VEGFR-1 ($V_{1-2-3-3}$).

[0018] In examples, the present disclosure provides that the VEGF binding portion consists essentially of IgG-like domains 2, 3 and 3 of VEGFR-1 (V_{2-3-3}).

[0019] The present disclosure provides pharmaceutical compositions comprising a therapeutically effective amount of an anti-VEGF agent as defined claims and a pharmaceutically acceptable excipient. The present disclosure provides methods of treating a VEGF-related disorder in a subject in need comprising administering to the subject a therapeutically effective amount of an anti-VEGF agent as defined. The anti-VEGF agent can be directly injected into the affected tissue or organ, such as an eye.

[0020] The present disclosure provides a method for treating ocular disease, wherein an anti-VEGF agent is administered locally to the eye at a dosage corresponding to a molar ratio of 2:1 compared to VEGF. The present disclosure provides a method for treating ocular disease, wherein an anti-VEGF agent is administered by intravitreal injection.

[0021] The present disclosure provides a method for treating ocular disease, wherein an anti-VEGF agent is administered intravitreally once every 10-30 weeks. In examples, the anti-VEGF agent is administered intravitreally once every 16 to 24 weeks. In examples, the treatment is continued for a period of at least one year.

[0022] The present disclosure provides a method for treating ocular disease comprising administering a therapeutically effective amount of an anti-VEGF agent locally into the eye wherein the treatment is effective to treat occult, minimally classic, and predominantly classic forms of wet macular degeneration, wherein the agent is a fusion protein.

[0023] The disclosure can be used to treat a wide variety of VEGF-related disorders including neovascular age related macular degeneration, choroidal neovascularization secondary to myopia, proliferative diabetic retinopathy, diabetic macular edema, retinal vascular obstruction such as retinal vein occlusion, ocular tumors,

von Hippel Lindau syndrome, retinopathy of prematurity, polypoid choroidal vasculopathy, or non-neoplastic disorders that benefit from anti-VEGF therapy.

[0024] The present disclosure provides a pharmaceutical formulation comprising an anti-VEGF agent in a pharmaceutically acceptable carrier formulation for local administration such as into the eye.

[0025] The present disclosure discloses novel constructs, wherein the constructs potentially neutralize the activity of VEGF while, at the same time, have strong heparin-binding characteristics.

[0025a] In one aspect, the present disclosure provides use of an anti-vascular endothelial growth factor (VEGF) agent in the manufacture of a medicament for treating a VEGF-related ophthalmic disorder in a subject in need thereof, wherein the anti-VEGF agent comprises a VEGF binding portion operatively linked to a Fc-IgG, wherein the VEGF binding portion comprises at least one VEGF binding domain that is an IgG-like domain 2 of VEGFR-1 and at least one VEGF binding domain that is an IgG-like domain 3 of VEGFR-1, and wherein:

(a) a first therapeutically effective amount of the anti-VEGF agent is formulated to be intravitreally administered to the subject and a second therapeutically effective amount of the anti-VEGF agent is to be intravitreally administered to the subject, and

(b) the second therapeutically effective amount of the anti-VEGF agent is formulated to be intravitreally administered to the subject from 10 to 30 weeks after the first therapeutically effective amount of the anti-VEGF agent is administered.

[0025b] In another aspect, the present disclosure provides use of an anti-VEGF agent in the formulation of a medicament for inhibiting intraocular neovascularization in a subject in need thereof, wherein the anti-VEGF agent comprises a VEGF binding portion operatively linked to a Fc-IgG, wherein the VEGF binding portion comprises at least one VEGF binding domain that is an IgG-like domain 2 of VEGFR-1 and at least one VEGF binding domain that is an IgG-like domain 3 of VEGFR-1, and wherein:

- (a) a first therapeutically effective amount of the anti-VEGF agent is formulated to be intravitreally administered to a subject and a second therapeutically effective amount of the anti-VEGF agent is to be intravitreally administered to the subject, and
- (b) the second therapeutically effective amount of the anti-VEGF agent is formulated to be intravitreally administered to the subject from 10 to 30 weeks after the first therapeutically effective amount of the anti-VEGF agent is intravitreally administered.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Figure 1 shows Immunoglobulin (Ig)-like domain (D) organization of VEGFR1 and of the Fc-fusion constructs designed in our study. Red label denotes heparin-binding domain. D2 is an indispensable binding element for VEGF and PlGF, responsible for ligand specificity²⁷. D3 plays an important role in binding affinity and stability^{27 28 30}. D3 of VEGFR1, but not D3 of VEGFR2, is a major heparin-binding site. V23 and aflibercept (EYLEA) differ only in D3, which is from VEGFR2 in aflibercept. D4 is also a heparin-binding site, implicated in receptor dimerization and homotypic interactions³⁰. Each construct is shown as a monomer for simplicity, but the recombinant proteins are dimers due the forced dimerization imposed by the Fc.

[0027] Figures 2A-2B show characterization of purified recombinant proteins. Figure 2A shows silver-stained SDS/PAGE (4-20% Tris) of our purified recombinant fusion proteins and EYLEA. 200 ng of each protein were subjected to electrophoresis under reducing conditions. Staining was performed by SilverQuest Silver Staining kit

(Invitrogen). Figure 2B shows an analytical size-exclusion chromatography (SEC) of V23, V233, V1233 and EYLEA, 25 µg of each. The Y-axis represents intensity of absorbance (A280) in milli-absorbance unit, and X-axis represents elution time in minutes.

[0028] Figure 3 shows IC₅₀ values of the inhibitors. Bovine choroidal endothelial cells were maintained as described in Approach For assays, cells are plated at low density. Inhibitors are then added at various concentrations as indicated in the figure. VEGF is added at the final concentration of 10ng/ml. Cell densities are evaluated after 5 days. IC₅₀ values were calculated using GraphPad Prism 5 (GraphPad Software, CA). Data

What is claimed is:

1. Use of an anti-vascular endothelial growth factor (VEGF) agent in the manufacture of a medicament for treating a VEGF-related ophthalmic disorder in a subject in need thereof, wherein the anti-VEGF agent comprises a VEGF binding portion operatively linked to a Fc-IgG, wherein the VEGF binding portion comprises at least one VEGF binding domain that is an IgG-like domain 2 of VEGFR-1 and at least one VEGF binding domain that is an IgG-like domain 3 of VEGFR-1, and wherein:
 - (a) a first therapeutically effective amount of the anti-VEGF agent is formulated to be intravitreally administered to the subject and a second therapeutically effective amount of the anti-VEGF agent is to be intravitreally administered to the subject, and
 - (b) the second therapeutically effective amount of the anti-VEGF agent is formulated to be intravitreally administered to the subject from 10 to 30 weeks after the first therapeutically effective amount of the anti-VEGF agent is administered.
2. The use of claim 1, wherein the second therapeutically effective amount of the anti-VEGF agent is formulated to be administered intravitreally within 16 to 24 weeks of administration of the first therapeutically effective amount.
3. The use of claim 1 or 2, wherein subsequent administrations of the therapeutically effective amount of the anti-VEGF agent are formulated to be administered to the subject intravitreally at 10 to 30 weeks intervals for a period of at least one year.
4. The use of any one of claims 1-3, wherein the VEGF binding portion consists essentially of IgG-like domains 1, 2, and 3 of VEGFR-1 (V₁₋₂₋₃), and wherein the anti-VEGF agent comprises amino acid residues 27 to 331 of SEQ ID NO: 1.
5. The use of any one of claims 1-3, wherein the VEGF binding portion consists essentially of IgG-like domains 2 and 3 of VEGFR-1 (V₂₋₃).
6. The use of claim 5, wherein the anti-VEGF agent comprises amino acid residues 27 to 459 of SEQ ID NO: 3.

7. The use of any one of claims 1-3, wherein the VEGF binding portion consists essentially of IgG-like domains 1, 2, 3 and 3 of VEGFR-1 (V₁₋₂₋₃₋₃), and wherein the anti-VEGF agent comprises amino acid residues 27 to 432 of SEQ ID NO: 5.
8. The use of any one of claims 1-3, wherein the VEGF binding portion consists essentially of IgG-like domains 2, 3 and 3 of VEGFR-1 (V₂₋₃₋₃), and wherein the anti-VEGF agent comprises amino acid residues 27 to 333 of SEQ ID NO: 7.
9. The use of any one of claims 1-8, wherein the first therapeutically effective amount or the second therapeutically effective amount of the anti-VEGF agent is from about 1 to about 10 mg.
10. The use of any one of claims 1-9, wherein the first therapeutically effective amount or the second therapeutically effective amount of the anti-VEGF agent is from about 3 to about 6 mg.
11. The use of any one of claims 1-10, wherein the VEGF-related ophthalmic disorder comprises neovascular age related macular degeneration, choroidal neovascularization secondary to myopia, proliferative diabetic retinopathy, diabetic macular edema, retinal vascular obstruction such as retinal vein occlusion, ocular tumors, von Hippel Lindau syndrome, retinopathy of prematurity, or polypoid choroidal vasculopathy.
12. Use of an anti-VEGF agent in the formulation of a medicament for inhibiting intraocular neovascularization in a subject in need thereof, wherein the anti-VEGF agent comprises a VEGF binding portion operatively linked to a Fc-IgG, wherein the VEGF binding portion comprises at least one VEGF binding domain that is an IgG-like domain 2 of VEGFR-1 and at least one VEGF binding domain that is an IgG-like domain 3 of VEGFR-1, and wherein:
 - (a) a first therapeutically effective amount of the anti-VEGF agent is formulated to be intravitreally administered to a subject and a second therapeutically effective amount of the anti-VEGF agent is to be intravitreally administered to the subject, and
 - (b) the second therapeutically effective amount of the anti-VEGF agent is formulated to be intravitreally administered to the subject from 10 to 30 weeks

after the first therapeutically effective amount of the anti-VEGF agent is intravitreally administered.

13. The use of claim 12, wherein the VEGF binding portion consists essentially of IgG-like domains 1, 2, and 3 of VEGFR-1 (V_{1-2-3}), and wherein the anti-VEGF agent comprises amino acid residues 27 to 331 of SEQ ID NO: 1.
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14. The use of claim 12, wherein the VEGF binding portion consists essentially of IgG-like domains 2 and 3 of VEGFR-1 (V_{2-3}).
15. The use of claim 14, wherein the anti-VEGF agent comprises amino acid residues 27 to 459 of SEQ ID NO: 3.
- 10 16. The use of claim 12, wherein the VEGF binding portion consists essentially of IgG-like domains 1, 2, 3 and 3 of VEGFR-1 ($V_{1-2-3-3}$), and wherein the anti-VEGF agent comprises amino acid residues 27 to 432 of SEQ ID NO: 5.
- 15 17. The use of claim 12, wherein the VEGF binding portion consists essentially of IgG-like domains 2, 3 and 3 of VEGFR-1 (V_{2-3-3}), and wherein the anti-VEGF agent comprises amino acid residues 27 to 333 of SEQ ID NO: 7.
18. The use of any one of claim 12-17, wherein the first therapeutically effective amount or the second therapeutically effective amount of the anti-VEGF agent is from about 1 to about 10 mg.
- 20 19. The use of any one of claim 12-18, wherein the first therapeutically effective amount or the second therapeutically effective amount of the anti-VEGF agent is from about 3 to about 6 mg.