

on VEGFR-3 the ligand specificity of VEGFR-1²⁰. Subsequent work documented the interaction between D2 and VEGF (or PlGF) by X-ray crystallography²¹⁻²³.

[0007] The initial studies led to the design of a construct with full VEGF binding characteristics, comprising the first three Ig-like Ds of VEGFR-1, fused to an Fc-IgG (Flt-1-3-IgG)²⁰. Flt-1-3-IgG showed a potent ability to neutralize VEGF, in vitro and in vivo²⁴⁻²⁷. However, the systemic half-life of this molecule was hampered by the presence of D3, which has significant heparin affinity due to the presence of clusters of basic residues, resulting in binding to HSPGs in various tissues. In 2002 Holash et al¹³ (US Patent No. 7,070,959) described an IgG fusion construct comprising of VEGFR-1 D2 (the binding element) and D3 of VEGFR2, which has much weaker heparin affinity compared to VEGFR-1 D3. This molecule, known today as aflibercept (marketed as EYLEA), was reported to have a longer half-life compared to Flt-(1-3-IgG) following systemic administration¹³, clearly an advantage for treatment aiming, for example, at oncological indications.

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] In examples, the present disclosure provides compositions and methods of treating conditions in which local direct administration of an anti-VEGF agent is beneficial, for example, treating and preventing endothelial cell proliferative conditions or angiogenesis, for example, in treating solid tumors, such as but not limited to, intracranial administration in glioblastomas.

[0010] In examples, 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. In examples, 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. In examples, 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. In examples, the present disclosure provides an anti-VEGF agent, wherein the anti-VEGF agent is a fusion protein with very low endotoxin levels.

[0011] In examples, the present disclosure provides an anti-VEGF agent, wherein the anti-VEGF agent is an IgG chimeric protein comprising elements of VEGF receptors. In examples, 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. In examples, 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. In examples, 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. In examples, 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. In examples, 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.

[0012] In one aspect, the present disclosure refers to 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 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.

[0013] In examples, the present disclosure provides that the VEGF binding portion consists essentially of IgG-like domains 1, 2, and 3 of VEGFR-1 (V_{1-2-3}). In examples, the anti-VEGF agent comprises amino acid sequence as defined in SEQ ID No: 1.

[0014] In examples, the present disclosure provides that the VEGF binding portion consists essentially of IgG-like domains 2 and 3 of VEGFR-1 (V_{2-3}). In examples, the anti-VEGF agent comprises amino acid sequence as defined in SEQ ID No: 3.

[0015] 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 ($V_{1-2-3-3}$). In examples, the anti-VEGF agent comprises amino acid sequence as defined in SEQ ID No: 5.

[0016] 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}). In examples, the the anti-VEGF agent comprises amino acid sequence as defined in SEQ ID No: 7.

[0017] In another aspect, the present disclosure refers to a pharmaceutical composition comprising a therapeutically effective amount of an anti-VEGF agent as disclosed herein and a pharmaceutically acceptable excipient. In examples, the present disclosure provides pharmaceutical compositions comprising a therapeutically effective amount of an anti-VEGF agent as defined claims and a pharmaceutically acceptable excipient. In examples, 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.

[0018] In yet another aspect, the present disclosure refers to use a therapeutically effective amount of an anti-VEGF agent as disclosed herein in the manufacture of a medicament for treating a VEGF-related disorder. In yet another aspect, the present disclosure refers to use of a therapeutically effective amount of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a VEGF-related disorder. In examples, 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. In examples, the present disclosure provides a method for treating ocular disease, wherein an anti-VEGF agent is administered by intravitreal injection. In examples, the present disclosure provides a method for treating ocular disease, wherein an anti-VEGF agent is administered every 4-6 weeks, and in other examples, the treatment is continued for a period of at least one year.

[0019] According to one example, 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.

[0020] In examples 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, colorectal cancer, lung cancer, cervical cancer, endometrial cancer, ovarian cancer, kidney cancer, schwannomas, gliomas, ependimomas, and neoplastic or non-neoplastic disorders that benefit from anti-VEGF therapy.

[0021] According to another example, 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.

[0022] In examples, 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0024] FIGURE 1 depicts a schematic representation of exemplary constructed fusion proteins with various Ig-like extracellular domains of VEGFR-1 (V) fused to Fc-IgG (Fc). The following constructs are shown: V₁₋₂₋₃-Fc; V₂₋₃-Fc; V₁₋₂₋₃₋₃-Fc; V₂₋₃₋₃-Fc; V₁₋₂₋₃₋₄-Fc; V₂₋₃₋₄-Fc; V₁₋₂₋₄-Fc and V₂₋₄-Fc.

WHAT IS CLAIMED IS:

1. 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.
2. The anti-VEGF agent of claim 1, wherein the anti-VEGF agent has a vitreous binding ability greater than aflibercept.
3. The anti-VEGF agent of claim 2, wherein the anti-VEGF agent has a vitreous bound VEGF-stimulated endothelial cell proliferation-inhibiting ability greater than aflibercept.
4. The anti-VEGF agent of claim 3, wherein the agent has an increased half-life *in vivo* compared to aflibercept.
5. The anti-VEGF agent of claim 4, wherein the VEGF binding portion consists essentially of IgG-like domains 1, 2, and 3 of VEGFR-1 (V₁₋₂₋₃).
6. The anti-VEGF agent of claim 5, wherein the anti-VEGF agent comprises amino acid sequence as defined in SEQ ID No: 1.
7. The anti-VEGF agent of claim 4, wherein the VEGF binding portion consists essentially of IgG-like domains 2 and 3 of VEGFR-1 (V₂₋₃).
8. The anti-VEGF agent of claim 5, wherein the anti-VEGF agent comprises amino acid sequence as defined in SEQ ID No: 3.
9. The anti-VEGF agent of claim 4, wherein the VEGF binding portion consists essentially of IgG-like domains 1, 2, 3 and 3 of VEGFR-1 (V₁₋₂₋₃₋₃).
10. The anti-VEGF agent of claim 5, wherein the anti-VEGF agent comprises amino acid sequence as defined in SEQ ID No: 5.
11. The anti-VEGF agent of claim 4, wherein the VEGF binding portion consists essentially of IgG-like domains 2, 3 and 3 of VEGFR-1 (V₂₋₃₋₃).

12. The anti-VEGF agent of claim 5, wherein the anti-VEGF agent comprises amino acid sequence as defined in SEQ ID No: 7.
13. A pharmaceutical composition comprising a therapeutically effective amount of an anti-VEGF agent of any of the preceding claims and a pharmaceutically acceptable excipient.
14. Use of a therapeutically effective amount of an anti-VEGF agent of any of claims 1-12 in the manufacture of a medicament for treating a VEGF-related disorder.
15. The use of claim 14, wherein the anti-VEGF agent is to be directly injected into the affected tissue or organ.
16. The use of claim 15, wherein the said affected tissue or organ is an eye.
17. The use of claim 14, wherein the VEGF-related disorder is selected from the group comprising 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, colorectal cancer, lung cancer, cervical cancer, endometrial cancer, ovarian cancer, kidney cancer, schwannomas, gliomas, ependimomas, and neoplastic or non-neoplastic disorders that benefits from anti-VEGF therapy.
18. Use of a therapeutically effective amount of the pharmaceutical composition of claim 13 in the manufacture of a medicament for treating a VEGF-related disorder.
19. The use of claim 18, wherein the anti-VEGF agent is to be directly injected into the affected tissue or organ.
20. The use of claim 20, wherein the said affected tissue or organ is an eye.
21. The use of claim 19, wherein the VEGF related disorder is selected from the group comprising 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, polypoid choroidal vasculopathy, von Hippel Lindau syndrome, retinopathy of prematurity, colorectal cancer, lung cancer, cervical cancer, ovarian cancer, endometrial cancer, kidney cancer, schwannomas, gliomas, ependimomas, and neoplastic or non-neoplastic disorders that benefit from anti-VEGF therapy.