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Document Date: 07/09/2010

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UTILITY PATENT APPLICATION TRANSMITTAL <i>(Only for new nonprovisional applications under 37 CFR 1.53(b))</i>	Attorney Docket No.	5050C
	First Inventor	Eric FURFINE
	Title	VEGF Antagonist Formulations for ..
	Express Mail Label No.	via EFS

APPLICATION ELEMENTS <i>See MPEP chapter 600 concerning utility patent application contents.</i>	ADDRESS TO: Commissioner for Patents P.O. Box 1450 Alexandria VA 22313-1450
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1. ☒ Fee Transmittal Form (e.g., PTO/SB/17) 2. ☐ Applicant claims small entity status. See 37 CFR 1.27. 3. ☒ Specification [Total Pages <u>22</u>] Both the claims and abstract must start on a new page (For information on the preferred arrangement, see MPEP 608.01(a)) 4. ☐ Drawing(s) (35 U.S.C. 113) [Total Sheets _____] 5. Oath or Declaration [Total Sheets _____] a. ☐ Newly executed (original or copy) b. ☒ A copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional with Box 18 completed) i. ☐ DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) name in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b). 6. ☒ Application Data Sheet. See 37 CFR 1.76 7. ☐ CD-ROM or CD-R in duplicate, large table or Computer Program (Appendix) ☐ Landscape Table on CD 8. Nucleotide and/or Amino Acid Sequence Submission (if applicable, items a. – c. are required) a. ☒ Computer Readable Form (CRF) b. Specification Sequence Listing on: i. ☐ CD-ROM or CD-R (2 copies); or ii. ☒ Paper c. ☒ Statements verifying identity of above copies	ACCOMPANYING APPLICATION PARTS 9. ☐ Assignment Papers (cover sheet & document(s)) Name of Assignee _____ 10. ☐ 37 CFR 3.73(b) Statement (when there is an assignee) ☐ Power of Attorney 11. ☐ English Translation Document (if applicable) 12. ☒ Information Disclosure Statement (PTO/SB/08 or PTO-1449) ☒ Copies of citations attached 13. ☐ Preliminary Amendment 14. ☐ Return Receipt Postcard (MPEP 503) (Should be specifically itemized) 15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed) 16. ☐ Nonpublication Request under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent. 17. ☐ Other: _____
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18. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76:

☒ Continuation
☐ Divisional
☐ Continuation-in-part (CIP)
of prior application No.: 12/560,885.....

Prior application information: Examiner LOCKARD, Jon M. Art Unit: 1647

19. CORRESPONDENCE ADDRESS

☒ The address associated with Customer Number: 26693 OR ☐ Correspondence address below					
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Address					
City	State	Zip Code			
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Signature		Date	2010-07-09
Name (Print/Type)	Joseph E. Zahner	Registration No. (Attorney/Agent)	48,224

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Electronic Patent Application Fee Transmittal

Application Number:				
Filing Date:				
Title of Invention:	VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION			
First Named Inventor/Applicant Name:	Eric FURFINE			
Filer:	Joseph Zahner			
Attorney Docket Number:	5050C			
Filed as Large Entity				
Utility under 35 USC 111(a) Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Utility application filing	1011	1	330	330
Utility Search Fee	1111	1	540	540
Utility Examination Fee	1311	1	220	220
Pages:				
Claims:				
Independent claims in excess of 3	1201	5	220	1100
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				2190

Electronic Acknowledgement Receipt

EFS ID:	7985892
Application Number:	12833417
International Application Number:	
Confirmation Number:	6438
Title of Invention:	VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION
First Named Inventor/Applicant Name:	Eric FURFINE
Customer Number:	26693
Filer:	Joseph Zahner
Filer Authorized By:	
Attorney Docket Number:	5050C
Receipt Date:	09-JUL-2010
Filing Date:	
Time Stamp:	15:25:53
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Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 2190
RAM confirmation Number	1646
Deposit Account	180650
Authorized User	

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		5050C_application.pdf	1078059	yes	18
			fdb3848f9e002500818630ce280fd213f5ce982c		

Multipart Description/PDF files in .zip description

	Document Description	Start	End
	Specification	1	14
	Claims	15	17
	Abstract	18	18

Warnings:

Information:

2	Sequence Listing	5050C_SEQ.pdf	74625	no	5
			0538f8bc07d126978cf8b508db8e7910d7c64617		

Warnings:

Information:

3	Application Data Sheet	ADS_5050C.pdf	389774	no	4
			cd3c9cf29aa57306d33a4084b2439c20f4fb2b0e		

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4	Oath or Declaration filed	declaration_5050C.pdf	299489	no	6
			45b21db6258d37a4a122d7e4d90a045cc69c1e64		

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5	Information Disclosure Statement (IDS) Filed (SB/08)	5050C_IDS.pdf	212730	no	3
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6	Foreign Reference	WO06104852A2.pdf	1096372	no	20
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Warnings:					
Information:					
7	Foreign Reference	WO06047325A1.pdf	2358828	no	46
			e69a93e7d5bc528a5db5c2e3f0076954612e7f4a		
Warnings:					
Information:					
8	Foreign Reference	WO05000895.pdf	2322424	no	38
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Warnings:					
Information:					
9	CRF Statement Paper and CRF are the same	5050C_821e.pdf	33141	no	1
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Warnings:					
Information:					
10	Computer Listing (text file)	5050Csequencelisting.txt	11770	no	0
Warnings:					
Information:					
11	Transmittal of New Application	5050C_transmittal3.pdf	102311	no	1
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Warnings:					
Information:					
12	Fee Worksheet (PTO-875)	fee-info.pdf	36067	no	2
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VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION

Cross-Reference to Related Applications

[0001] This application is a continuation application of U.S. Patent Application Serial No. 12/560,885, filed 16 September 2009, which is a divisional application of U.S. Patent Application Serial No. 11/818,463, filed 14 June 2007, which issued as U.S. Patent No. 7,608,261 on 27 October 2009, which claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No.: 60/814,484, filed 16 June 2006, which applications are each hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present invention is directed to pharmaceutical formulations suitable for intravitreal administration comprising agents capable of inhibiting vascular endothelial growth factor (VEGF), and to methods for making and using such formulations. The invention includes liquid pharmaceutical formulations having increased stability, as well as formulations that may be lyophilized and reconstituted for intravitreal administration.

Statement of Related Art

[0003] Vascular endothelial growth factor (VEGF) expression is nearly ubiquitous in human cancer, consistent with its role as a key mediator of tumor neoangiogenesis. Blockade of VEGF function, by binding to the molecule or its VEGFR-2 receptor, inhibits growth of implanted tumor cells in multiple different xenograft models (see, for example, Gerber et al. (2000) Cancer Res. 60:6253-6258). A soluble VEGF-specific fusion protein antagonist, termed a "VEGF-trap" has been described (Kim et al. (2002) Proc. Natl. Acad. Sci. USA 99:11399-404; Holash et al. (2002) Proc. Natl. Acad. Sci. USA 99:11393-8), which applications are specifically incorporated by reference in their entirety.

[0004] Ophthalmic formulations are known, see for example, U.S. 7,033,604 and 6,777,429. An ophthalmic formulation of a VEGF antibody is described in U.S. 6,676,941.

[0005] Lyophilization (freeze drying under controlled conditions) is commonly used for long-term storage of proteins. The lyophilized protein is substantially resistant to degradation, aggregation, oxidation, and other degenerative processes while in the freeze-dried state (see, for example, U.S. 6,436,897).

BRIEF SUMMARY OF THE INVENTION

[0006] Stable formulations of a VEGF-specific fusion protein antagonist are provided.

Pharmaceutically acceptable formulations are provided that comprise a VEGF "trap" antagonist with a pharmaceutically acceptable carrier. In specific embodiments, liquid and lyophilized formulations are provided.

[0007] In a first aspect, a stable liquid ophthalmic formulation of a VEGF-specific fusion protein antagonist is provided, comprising a fusion protein that comprises a receptor component consisting essentially of an immunoglobulin-like (Ig) domain 2 of a first VEGF receptor and Ig domain 3 of a second VEGF receptor, and a multimerizing component (also termed a "VEGF trap"). In a specific embodiment of the VEGF-specific fusion protein antagonist, the first VEGF receptor is Flt1 and the second VEGF receptor is Flk1 or Flt4. In a more specific embodiment the fusion protein has the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. Preferably, the VEGF antagonist is a dimer comprising two fusion proteins of SEQ ID NO:4.

[0008] In one aspect, a stable liquid ophthalmic formulation is provided that comprises 1-100 mg/ml VEGF-specific fusion protein antagonist, 0.01-5% of one or more organic co-solvent(s), 30-150 mM of one or more tonicity agent(s), 5-40 mM of a buffering agent, and optionally, 1.0-7.5% of a stabilizing agent, pH between about 5.8-7.0.

[0009] In one or more specific embodiments, the organic co-solvent may be polysorbate, for example, polysorbate 20 or polysorbate 80, polyethylene glycol (PEG), for example, PEG 3350, or propylene glycol, or a combination thereof; the tonicity agent may be, for example, sodium chloride or potassium chloride; the stabilizing agent may be sucrose, sorbitol, glycerol, trehalose, or mannitol; and the buffering agent may be, for example, phosphate buffer. In a specific embodiment, the phosphate buffer is a sodium phosphate buffer.

[0010] In various embodiments, the organic co-solvent is polysorbate and/or PEG, the stabilizing agent is sucrose, the buffering agent is phosphate buffer, and the tonicity agent is sodium chloride.

[0011] More specifically, the stable liquid ophthalmic formulation comprises about 40-50 mg/ml of the VEGF antagonist (SEQ ID NO:4), about 10 mM phosphate buffer, 0.01-3% polysorbate and/or PEG, 40-135 mM sodium chloride, and optionally 5.0% sucrose, pH about 6.2-6.3.

[0012] In a specific preferred embodiment, the stable liquid ophthalmic formulation comprises about 50 mg/ml of the VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 50 mM sodium chloride, 0.1% polysorbate, and 5% sucrose, pH about 6.2-6.3.

[0013] In a specific preferred embodiment, the stable liquid ophthalmic formulation comprises about 50 mg/ml of the VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 50 mM sodium chloride, 3% PEG, and 5% sucrose, pH about 6.2-6.3.

[0014] In a specific preferred embodiment, the stable liquid ophthalmic formulation comprises about 40 mg/ml of the VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 40 mM sodium chloride, 0.03% polysorbate, and 5% sucrose, pH about 6.2-6.3.

[0015] In a specific preferred embodiment, the stable liquid ophthalmic formulation comprises about 40 mg/ml of the VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 135 mM sodium chloride, and 0.03% polysorbate, pH about 6.2-6.3.

[0016] In another aspect, a stable liquid ophthalmic formulation is provided that comprises 1-100 mg/ml VEGF-specific fusion protein antagonist; 0.01-5% of one or more organic co-solvent(s); 5-40 mM of a buffering agent; and optionally 30-150 mM of one or more tonicity agent(s) and/or 1.0-7.5% of a stabilizing agent; having a pH between about 5.8-7.0.

[0017] In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 10 to about 80 mg/ml. In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 10, about 20, about 30, about 40, about 50, about 60, about 70, or about 80 mg/ml. In a preferred embodiment, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 40 mg/ml.

[0018] In another embodiment, the stabilizing agent is selected from one or more of sucrose, sorbitol, glycerol, trehalose, and mannitol.

[0019] In another embodiment, the organic co-solvent is selected from one or more of polysorbate, for example, polysorbate 20 or polysorbate 80, polyethylene glycol (PEG), for example, PEG 3350, and propylene glycol.

[0020] In another embodiment, the buffer is a phosphate buffer, for example, sodium phosphate.

[0021] In another embodiment, the tonicity agent is a salt, for example, sodium chloride.

[0022] In one embodiment, the stable liquid ophthalmic formulation comprises 10 mM sodium phosphate buffer, about 0.03 to about 0.1% polysorbate and/or about 3% PEG or propylene glycol, about 40 mM sodium chloride, and about 5% sucrose. In a specific embodiment, the stable liquid ophthalmic formulation comprises 10 mM sodium phosphate buffer, about 0.03% polysorbate, about 40 mM sodium chloride, and about 5% sucrose. In another specific embodiment, the pH of the formulation is about 6.2 to about 6.3. In another specific embodiment, the pH is achieved by mixing mono- and dibasic sodium phosphate to the desired pH without acid/base titration.

[0023] In a specific embodiment, the stable liquid ophthalmic formulation consists essentially of a VEGF antagonist (SEQ ID NO:4) at 40 mg/ml, 10 mM sodium phosphate buffer, polysorbate at 0.03%, sodium chloride at 40 mM, and sucrose at 5%, pH 6.2-6.3.

[0024] In another aspect, a stable liquid ophthalmic formulation is provided that comprises about 10 to about 80 mg/ml VEGF antagonist, about 10 mM sodium phosphate buffer, about 0.03%

polysorbate, and about 135 mM sodium chloride, pH 6.2 to 6.3.

[0025] In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 10 to about 80 mg/ml. In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 10, about 20, about 30, about 40, about 50, about 60, about 70, or about 80 mg/ml. In a specific embodiment, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 40 mg/ml.

[0026] In one embodiment, the stable liquid ophthalmic formulation comprises 40 mg/ml of VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 0.03% polysorbate, and 135 mM sodium chloride at pH 6.2-6.3. In a specific embodiment, the stable liquid ophthalmic formulation consists essentially of 40 mg/ml of VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 0.03% polysorbate, and 135 mM sodium chloride at pH 6.2-6.3.

[0027] In another aspect, a lyophilizable formulation of a VEGF antagonist is provided, wherein upon lyophilization followed by reconstitution, a stable liquid ophthalmic formulation as described herein is obtained.

[0028] In another aspect, a lyophilizable formulation of a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist is provided, comprising 5-50 mg/ml of the VEGF antagonist, 5-25 mM buffer, such as phosphate buffer, 0.01 to 0.15% of one or more of an organic co-solvent, such as polysorbate, propylene glycol and/or PEG, and optionally 1-10% of a stabilizing agent such as sucrose, sorbitol, trehalose, glycerol, or mannitol, pH about 5.8-7.0. In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at about 5, about 10, about 20, about 30, or about 40 mg/ml. In a specific embodiment, the lyophilizable ophthalmic formulation of the invention comprises 20 mg/ml of the VEGF antagonist, 10 mM sodium phosphate buffer, 0.03% polysorbate, 0.1% PEG, and 2.5% sucrose, pH about 6.2-6.3. In further embodiments, the lyophilizable formulation further comprises sodium chloride. In a specific embodiment, the sodium chloride is present at a concentration of about 20 mM. In another specific embodiment, the sodium chloride is present at a concentration of about 67.5 mM.

[0029] In another specific embodiment, the lyophilizable ophthalmic formulation of the invention comprises 20 mg/ml of the VEGF antagonist, 5 mM sodium phosphate buffer, 0.015% polysorbate, 20 mM sodium chloride, and 2.5% sucrose, pH about 6.2-6.3.

[0030] In another embodiment, the lyophilizable ophthalmic formulation comprises 5 mg/ml, 10 mg/ml, or 40 mg/ml VEGF antagonist, 5 mM sodium phosphate buffer, 0.015% polysorbate, 20 mM sodium chloride, and 2.5% sucrose, at pH 6.2-6.3. In a specific embodiment, the lyophilizable ophthalmic formulation consists essentially of 5 mg/ml, 10 mg/ml, or 40 mg/ml VEGF antagonist (SEQ ID NO:4), 5 mM sodium phosphate buffer, 0.015% polysorbate, 20 mM sodium chloride, and

2.5% sucrose, at pH 6.2-6.3.

[0031] In another specific embodiment, the lyophilizable ophthalmic formulation comprises 20 mg/ml of the VEGF antagonist, 5 mM sodium phosphate buffer, 0.015% polysorbate, and 67.5 mM sodium chloride, pH about 6.2-6.3. In a more specific embodiment, the lyophilizable ophthalmic formulation consists essentially of 20 mg/ml of the VEGF antagonist (SEQ ID NO:4), 5 mM sodium phosphate buffer, 0.015% polysorbate, and 67.5 mM sodium chloride, pH 6.2-6.3.

[0032] In another specific embodiment, the lyophilizable ophthalmic formulation comprises 5 mg/ml, 10 mg/ml, or 40 mg/ml VEGF antagonist, 5 mM sodium phosphate buffer, 0.015% polysorbate, and 67.5 mM sodium chloride, pH about 6.2-6.3. In a more specific embodiment, the lyophilizable ophthalmic formulation consists essentially of 5 mg/ml, 10 mg/ml, or 40 mg/ml VEGF antagonist (SEQ ID NO:4), 5 mM sodium phosphate buffer, 0.015% polysorbate, and 67.5 mM sodium chloride, pH about 6.2-6.3.

[0033] Generally, the reconstituted formulation is about 2 times the concentration of the pre-lyophilized formulation, e.g., a 20 mg fusion protein/ml pre-lyophilized formulation is reconstituted to a final formulation of 40 mg fusion protein/ml.

[0034] Generally, the lyophilized formulation is reconstituted with sterile water suitable for injection. In one embodiment, the reconstitution liquid is bacteriostatic water.

[0035] In another aspect, the invention features a method of producing a lyophilized formulation of a VEGF-specific fusion protein antagonist, comprising subjecting the lyophilizable formulation of the invention to lyophilization to generate a lyophilized formulation. The lyophilized formulation may be lyophilized by any method known in the art for lyophilizing a liquid.

[0036] In another related aspect, the invention features a method of producing a reconstituted lyophilized formulation of a VEGF antagonist, comprising reconstituting the lyophilized formulation of the invention to a reconstituted formulation. In one embodiment, the reconstituted formulation is twice the concentration of the pre-lyophilized formulation, e.g., the method of the invention comprises: (a) producing a pre-lyophilized formulation of a VEGF-specific fusion protein antagonist, (b) subjecting the pre-lyophilized formulation of step (a) to lyophilization; and (c) reconstituting the lyophilized formulation of step (b).

[0037] The invention further features ophthalmic formulations provided in a pre-filled syringe or vial, particularly suitable for intravitreal administration.

[0038] Other objects and advantages will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION OF THE INVENTION

[0039] The present invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting unless indicated, since the scope of the present invention will be limited only by the appended claims.

[0040] Unless stated otherwise, all technical and scientific terms and phrases used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference.

General Description

[0041] Safe handling and administration of formulations comprising proteins represent significant challenges to pharmaceutical formulators. Proteins possess unique chemical and physical properties that present stability problems: a variety of degradation pathways exist for proteins, implicating both chemical and physical instability. Chemical instability includes deamination, aggregation, clipping of the peptide backbone, and oxidation of methionine residues. Physical instability encompasses many phenomena, including, for example, aggregation and/or precipitation.

[0042] Chemical and physical stability can be promoted by removing water from the protein. Lyophilization (freeze-drying under controlled conditions) is commonly used for long-term storage of proteins. The lyophilized protein is substantially resistant to degradation, aggregation, oxidation, and other degenerative processes while in the freeze-dried state. The lyophilized protein may be reconstituted with water optionally containing a bacteriostatic preservative (e.g., benzyl alcohol) prior to administration.

Definitions

[0043] The term "carrier" includes a diluent, adjuvant, excipient, or vehicle with which a composition is administered. Carriers can include sterile liquids, such as, for example, water and oils, including oils of petroleum, animal, vegetable or synthetic origin, such as, for example, peanut oil, soybean oil, mineral oil, sesame oil and the like.

[0044] The term "excipient" includes a non-therapeutic agent added to a pharmaceutical composition to provide a desired consistency or stabilizing effect. Suitable pharmaceutical excipients include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk,

silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

[0045] The term "lyophilized" or "freeze-dried" includes a state of a substance that has been subjected to a drying procedure such as lyophilization, where at least 90% of moisture has been removed.

VEGF Antagonists

[0046] A VEGF antagonist is a compound capable of blocking or inhibiting the biological action of vascular endothelial growth factor (VEGF), and includes fusion proteins capable of trapping VEGF. In a preferred embodiment, the VEGF antagonist is the fusion protein of SEQ ID NO:2 or 4; more preferably, SEQ ID NO:4. In specific embodiments, the VEGF antagonist is expressed in a mammalian cell line such as a CHO cell and may be modified post-translationally. In a specific embodiment, the fusion protein comprises amino acids 27-457 of SEQ ID NO:4 and is glycosylated at Asn residues 62, 94, 149, 222 and 308. Preferably, the VEGF antagonist is a dimer composed of two fusion proteins of SEQ ID NO:4.

[0047] The VEGF antagonist of the methods and formulations of the invention can be prepared by any suitable method known in the art, or that comes to be known. The VEGF antagonist is preferably substantially free of protein contaminants at the time it is used to prepare the pharmaceutically acceptable formulation. By "substantially free of protein contaminants" is meant, preferably, that at least 90 % of the weight of protein of the VEGF-specific fusion protein antagonist preparation used for making a formulation is VEGF fusion protein antagonist protein, more preferably at least 95%, most preferably at least 99%. The fusion protein is preferably substantially free of aggregates. "Substantially free of aggregates" means that at least 90% of the weight of fusion protein is not present in an aggregate at the time the fusion protein is used to prepare the pharmaceutically effective formulation. Unless stated otherwise, the phosphates employed are sodium phosphates and a desired buffering pH is achieved by mixing appropriate amounts of mono- and dibasic sodium phosphate.

Stable Liquid Ophthalmic Formulations

[0048] In one aspect, the invention provides a stable pharmaceutically acceptable formulation comprising a VEGF antagonist, wherein the formulation is a liquid formulation suitable for ophthalmic use. Preferably, the liquid formulation comprises a pharmaceutically effective amount of the VEGF antagonist. The formulation can also comprise one or more pharmaceutically acceptable carriers, buffers, tonicity agents, stabilizers, and/or excipients. An example of a pharmaceutically

acceptable liquid formulation comprises a VEGF antagonist in a pharmaceutically effective amount, a buffer, an organic co-solvent such as polysorbate, a tonicity agent such as NaCl, and optionally, a stabilizer such as sucrose or trehalose.

[0049] Stability is determined in a number of ways at specified time points, including determination of pH, visual inspection of color and appearance, determination of total protein content by methods known in the art, e.g., UV spectroscopy, and purity is determined by, for example, SDS-PAGE, size-exclusion HPLC, bioassay determination of activity, isoelectric focusing, and isoaspartate quantification. In one example of a bioassay useful for determining VEGF antagonist activity, a BAF/3 VEGFR1/EPOR cell line is used to determine VEGF₁₆₅ binding by the VEGF antagonist of the invention.

[0050] Liquid formulations can be stored in an oxygen-deprived environment. Oxygen-deprived environments can be generated by storing the formulations under an inert gas such as, for example, nitrogen or argon. Liquid formulations are preferably stored at about 5°C.

Ophthalmic Lyophilized Formulations

[0051] In one aspect of the invention, an ophthalmically acceptable formulation comprising a VEGF antagonist is provided, wherein the formulation is a lyophilizable formulation. Lyophilizable formulations can be reconstituted into solutions, suspensions, emulsions, or any other suitable form for administration or use. Lyophilizable formulations are typically first prepared as liquids, then frozen and lyophilized. The total liquid volume before lyophilization can be less, equal to, or more than, the final reconstituted volume of the lyophilized formulation. The lyophilization process is well known to those of ordinary skill in the art, and typically includes sublimation of water from a frozen formulation under controlled conditions.

[0052] Lyophilized formulations can be stored at a wide range of temperatures. Lyophilized formulations may be stored below 25°C, for example, refrigerated at 2-8°C, or at room temperature (e.g., approximately 25°C). Preferably, lyophilized formulations are stored below about 25°C, more preferably, at about 4-20°C; below about 4°C; below about -20°C; about -40°C; about -70°C, or about -80°C. Stability of the lyophilized formulation may be determined in a number of ways known to the art, for example, by visual appearance of the cake and/or by moisture content.

[0053] Lyophilized formulations are typically reconstituted for use by addition of an aqueous solution to dissolve the lyophilized formulation. A wide variety of aqueous solutions can be used to reconstitute a lyophilized formulation. Preferably, lyophilized formulations are reconstituted using water. Lyophilized formulations are preferably reconstituted with a solution consisting essentially of water (e.g., USP WFI, or water for injection) or bacteriostatic water (e.g., USP WFI with 0.9%

benzyl alcohol). However, solutions comprising buffers and/or excipients and/or one or more pharmaceutically acceptable carries can also be used.

[0054] Freeze-dried or lyophilized formulations are typically prepared from liquids, that is, from solutions, suspensions, emulsions, and the like. Thus, the liquid that is to undergo freeze-drying or lyophilization preferably comprises all components desired in a final reconstituted liquid formulation. As a result, when reconstituted, the freeze-dried or lyophilized formulation will render a desired liquid formulation upon reconstitution.

EXAMPLES

[0055] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only to the appended claims.

[0056] As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Thus for example, a reference to “a method” includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0057] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

Example 1. Stability of 50 mg/ml VEGF Trap Liquid Formulation Stored at 5°C in 3 ml Glass Vials.

[0058] An ophthalmic liquid formulation containing 50 mg/ml VEGF Trap (SEQ ID NO:4), 10 mM phosphate, 50 mM NaCl, 0.1% polysorbate 20, 5% sucrose, and pH 6.25, was stored at 5 °C in 3 ml glass vials and samples tested at 3, 6, 9, 12, 18 and 24 months. Stability was determined by SE-HPLC. The results are shown in Table 1. Turbidity was measured at OD₄₀₅ nm; and percent recovered protein and purity by size exclusion HPLC.

Table 1. Stability of 50 mg/ml VEGF Trap Protein (VGFT-SS065)

Months	Visual Appearance	Turbidity (OD ₄₀₅ nm)	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.2	100	98.8
3	Pass	0.00	6.2	101	98.7
6	Pass	0.01	6.3	100	98.3
9	Pass	0.01	6.3	101	98.3
12	Pass	0.01	6.3	104	98.4
18	Pass	0.01	6.3	96	98.1
24	Pass	0.01	6.3	105	98.1

Example 2. Stability of 50 mg/ml VEGF Trap Liquid Formulation Stored at 5°C in 3 ml Glass Vials.

[0059] A liquid formulation containing 50 mg/ml VEGF Trap (SEQ ID NO:4), 10 mM phosphate, 50 mM NaCl, 3% polyethylene glycol 3350, 5% sucrose, and pH 6.25, was stored at 5 °C in 3 ml glass vials and samples tested at 3, 6, 9, 12, 18 and 24 months. Stability results are shown in Table 2. Turbidity, percent recovered protein and purity was determined as described above.

Table 2. Stability of 50 mg/ml VEGF Trap Protein (VGFT-SS065)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.2	100	98.9
3	Pass	0.00	6.1	104	98.5
6	Pass	0.01	6.3	99	98.3
9	Pass	0.00	6.3	102	97.6
12	Pass	0.01	6.3	103	98.0
18	Pass	0.00	6.3	113	97.7
24	Pass	0.00	6.2	106	97.6

Example 3. Stability of 40 mg/ml VEGF Trap Liquid Formulation Stored at 5°C in 3 ml Glass Vials.

[0060] A liquid formulation containing 40 mg/ml VEGF Trap (SEQ ID NO:4), 10 mM phosphate, 40

mM NaCl, 0.03% polysorbate 20, 5% sucrose, and pH 6.3, was stored at 5 °C in 3 ml glass vials and samples tested at 0.5, 1, 2, 3, and 4 months. Stability results are shown in Table 3. Turbidity, percent recovered protein and purity was determined as described above.

Table 3. Stability of 40 mg/ml VEGF Trap Protein (VGFT-SS207)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.3	100	99.5
0.5	Pass	0.00	6.3	99	99.4
1	Pass	0.00	6.2	98	99.5
2	Pass	0.00	6.2	95	99.2
3	Pass	0.01	6.4		
4	Pass	0.01	6.3		

Example 4. Stability of 40 mg/ml VEGF Trap Liquid Formulation Stored at 5°C in Pre-filled Glass Syringe.

[0061] A liquid formulation containing 40 mg/ml VEGF trap (SEQ ID NO:4), 10 mM phosphate, 40 mM NaCl, 0.03% polysorbate 20, 5% sucrose, and pH 6.3, was stored at 5 °C in 1 ml prefilled luer glass syringe with 4023/50 FLUROTEC™ coated (elastomer coated) plunger and samples tested at 0.5, 1, 2, 3, and 4 months. Stability results are shown in Table 4. Turbidity, percent recovered protein and purity was determined as described above.

Table 4. Stability of 40 mg/ml VEGF Trap Protein (VGFT-SS207)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.3	100	99.4
0.5	Pass	0.00	6.3	100	99.3
1	Pass	0.00	6.3	100	99.4
2	Pass	0.00	6.3	97	99.1
3	Pass	0.01	6.4		
4	Pass	0.01	6.3		

Example 5. Stability of 40 mg/ml VEGF Trap Liquid Formulation Stored at 5°C in 3 ml Glass Vials.

[0062] A liquid formulation containing 40 mg/ml VEGF trap (SEQ ID NO:4), 10 mM phosphate, 135 mM NaCl, 0.03% polysorbate 20, and pH 6.3, was stored at 5 °C in 3 ml glass vials and samples tested at 0.5, 1, 2, 3, and 4 months. Stability results are shown in Table 5. Turbidity, percent recovered protein and purity was determined as described above.

Table 5. Stability of 40 mg/ml VEGF Trap Protein (VGFT-SS203)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.3	100	99.3
0.5	Pass	0.00	6.2	87	99.2
1	Pass	0.00	6.2	88	99.1
2	Pass	0.00	6.3	103	99.2
3	Pass	0.00	6.3	88	99.0
4	Pass	0.00	6.2	85	98.9
5	Pass	0.00	6.3	84	99.0

Example 6. Stability of 40 mg/ml VEGF Trap Liquid Formulation Stored at 5°C in 1 ml Pre-filled Glass Syringe.

[0063] A liquid formulation containing 40 mg/ml VEGF trap (SEQ ID NO:4), 10 mM phosphate, 135 mM NaCl, 0.03% polysorbate 20, and pH 6.3, was stored at 5 °C in 1 ml prefilled glass luer syringe with 4023/50 FLUROTEC™ coated (elastomer coated) plunger and samples tested at 0.5, 1, 2, 3, 4, and 5 months. Stability results are shown in Table 6. Turbidity, percent recovered protein and purity was determined as described above.

Table 6. Stability of 40 mg/ml VEGF Trap Protein (VGFT-SS203)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.3	100	99.2
0.5	Pass	0.01	6.3	101	99.2
1	Pass	0.00	6.3	101	99.2
2	Pass	0.00	6.3	-	-
3	Pass	0.01	6.3	102	99.1
4	Pass	0.01	6.3	103	98.8
5	Pass	0.00	6.3	99	98.9

Example 7. Stability of Lyophilized 20 mg/ml VEGF Trap Formulation Stored at 5°C in 3 ml Glass Vials and Reconstituted to 40 mg/ml.

[0064] 0.8 ml of a liquid formulation containing 20 mg/ml VEGF trap (SEQ ID NO:4), 5 mM phosphate, 20 mM NaCl, 0.015% polysorbate 20, 2.5% sucrose, and pH 6.3, were lyophilized in 3 ml glass vials. Samples were stored at 5°C and tested at 1, and 2 months. VEGF trap was reconstituted to a final concentration of 40 mg/ml VEGF Trap (final volume of 0.4 ml). Stability results are shown in Table 7 (t = time in months; * = visual appearance; **=reconstitution time). Turbidity, percent recovered protein and purity was determined as described above.

Table 7. Stability of Lyophilized 20 mg/ml VEGF Trap Protein (VGFT-SS216)

t	Vis. App.*	Recon. Time** (min)	Vis. App.* Reconst'd Liquid	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Config.
0	Pass	0.6	Pass	0.00	6.3	100	99.5
1	Pass	0.6	Pass	0.01	6.3	106	99.4
2	Pass	0.4	Pass	0.01	6.2	103	99.3

Example 8. Stability of Lyophilized 20 mg/ml VEGF Trap Formulation Stored at 5°C in 3 ml Glass Vials.

[0065] 0.8 ml of a liquid formulation containing 20 mg/ml VEGF trap (SEQ ID NO:4), 5 mM phosphate, 67.5 mM NaCl, 0.015% polysorbate 20, and pH 6.3, were lyophilized in 3 ml glass vials. Samples were stored at 5°C and tested at 1, 2, and 3 months. VEGF trap was reconstituted to a

final concentration of 40 mg/ml VEGF trap (final volume of 0.4 ml). Stability results are shown in Table 8 (t = time in months; * = visual appearance; **=reconstitution time).

Table 8. Stability of Lyophilized 20 mg/ml VEGF Trap Protein (VGFT-SS216)

t	Vis. App.*	Recon. Time** (min)	Vis. App. Reconst'd Liquid	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Config.
0	Pass	0.7	Pass	0.00	6.3	100	99.0
1	Pass	0.7	Pass	0.01	6.2	105	98.9
2	Pass	0.4	Pass	0.01	6.2	103	98.9

We claim:

1. A stable liquid ophthalmic formulation of a vascular endothelial growth factor (VEGF) antagonist, comprising:

- (a) between 50 mg/ml to about 10 mg/ml of a VEGF antagonist of SEQ ID NO:4;
- (b) about 0.03% to about 0.1% polysorbate;
- (c) about 5-40 mM of sodium phosphate buffer, pH of about 5.8 to about 7.0; and
- (d) either:
 - (i) about 5% sucrose and about 40 to about 50 mM NaCl, or
 - (ii) about 135 mM NaCl,

wherein at least 99% of the VEGF antagonist is present in native conformation following storage at 5°C for 2 months as measured by size exclusion chromatography.

2. The formulation of claim 1, comprising between 50 mg/ml to about 10 mg/ml of the VEGF antagonist, about 10 mM sodium phosphate buffer, about 40 mM NaCl, about 0.03% polysorbate, and about 5% sucrose, pH about 6.2-6.3.

3. The formulation of claim 2, comprising about 10 to about 40 mg/ml of the VEGF antagonist.

4. The formulation of claim 3, comprising VEGF antagonist at a concentration of about 10 mg/ml.

5. The formulation of claim 3, comprising VEGF antagonist at a concentration of about 40 mg/ml.

6. The formulation of claim 1, comprising between 50 mg/ml to about 10 mg/ml of the VEGF antagonist, about 10 mM sodium phosphate buffer, about 135 mM NaCl and about 0.03% polysorbate, pH about 6.2-6.3.

7. The formulation of claim 6, comprising about 10 to about 40 mg/ml of the VEGF antagonist.

8. The formulation of claim 7, comprising VEGF antagonist at a concentration of about 10 mg/ml.

9. The formulation of claim 7, comprising VEGF antagonist at a concentration of about 40 mg/ml.
10. A pre-filled syringe suitable for intravitreal administration comprising the formulation of claim 1.
11. A pre-filled syringe suitable for intravitreal administration comprising the formulation of claim 4.
12. A pre-filled syringe suitable for intravitreal administration comprising the formulation of claim 5.
13. A pre-filled syringe suitable for intravitreal administration comprising the formulation of claim 6.
14. A pre-filled syringe suitable for intravitreal administration comprising the formulation of claim 8.
15. A pre-filled syringe suitable for intravitreal administration comprising the formulation of claim 9.
16. A non-liquid formulation of a vascular endothelial growth factor (VEGF) antagonist made according to the steps of:
- (a) combining in a liquid solution the following excipients:
 - (i) about 5-50 mg/ml of the VEGF antagonist, comprising the amino acid sequence of SEQ ID NO:4;
 - (ii) about 5-25 mM of sodium phosphate buffer, pH about 5.8-7.0;
 - (iii) about 0.01-0.15% of an organic co-solvent, selected from the group consisting of polysorbate, polyethylene glycol (PEG), propylene glycol, and a combination thereof; and
 - (iv) about 1-10% of a stabilizing agent selected from the group consisting of sucrose, sorbitol, glycerol, trehalose, and mannitol; and
 - (b) lyophilizing the combination of step (a),
- wherein at least about 99% of the VEGF antagonist is present in native conformation as

measured by size exclusion chromatography following storage at 5°C for 2 months.

17. The non-liquid formulation of claim 16, wherein about 20 mg/ml of the VEGF antagonist, about 5 mM sodium phosphate buffer, about 0.015% polysorbate, about 2.5% sucrose, and about 20 mM sodium chloride, pH about 6.2-6.3 are combined at step (a).

18. The non-liquid formulation of claim 16, wherein about 20 mg/ml of the VEGF antagonist, about 5 mM sodium phosphate buffer, about 0.015% polysorbate, and about 67.5 mM sodium chloride, pH about 6.2-6.3 are combined at step (a).

19. The non-liquid formulation of claim 16, wherein the formulation can be reconstituted in at least about 0.7 minutes or less.

ABSTRACT

Ophthalmic formulations of a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist are provided suitable for intravitreal administration to the eye. The ophthalmic formulations include a stable liquid formulation and a lyophilizable formulation. Preferably, the protein antagonist has the amino acid sequence shown in SEQ ID NO:4.

SEQUENCE LISTING

<110> Furfine, Eric
Dix, Daniel
Graham, Kenneth
Frye, Kelly

<120> VEGF Antagonist Formulations Suitable For Intravitreal Administration

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Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	
				405					410					415		
Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	
			420					425					430			
Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	
		435					440					445				
Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys							
	450					455										

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	5050C
		Application Number	
Title of Invention	VEGF Antagonist Formulations for Intravitreal Administration		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2

- ☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Applicant Information:

Applicant 1					
Applicant Authority		☒ Inventor		☐ Legal Representative under 35 U.S.C. 117	
				☐ Party of Interest under 35 U.S.C. 118	
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Eric		FURFINE		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Concord	State/Province	MA	Country of Residence	US
Citizenship under 37 CFR 1.41(b)		US			
Mailing Address of Applicant:					
Address 1		315 Harrington Avenue			
Address 2					
City	Concord	State/Province	MA		
Postal Code	01742	Country	US		
Applicant 2					
Applicant Authority		☒ Inventor		☐ Legal Representative under 35 U.S.C. 117	
				☐ Party of Interest under 35 U.S.C. 118	
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Daniel		DIX		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	LaGrangeville	State/Province	NY	Country of Residence	US
Citizenship under 37 CFR 1.41(b)		US			
Mailing Address of Applicant:					
Address 1		55 Memory Trail			
Address 2					
City	LaGrangeville	State/Province	NY		
Postal Code	12540	Country	US		
Applicant 3					
Applicant Authority		☒ Inventor		☐ Legal Representative under 35 U.S.C. 117	
				☐ Party of Interest under 35 U.S.C. 118	
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Kenneth	S.	GRAHAM		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Pleasant Valley	State/Province	NY	Country of Residence	US

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	5050C
		Application Number	
Title of Invention	VEGF Antagonist Formulations for Intravitreal Administration		

Citizenship under 37 CFR 1.41(b)		US	
Mailing Address of Applicant:			
Address 1	218 Robbout Road		
Address 2			
City	Pleasant Valley	State/Province	NY
Postal Code	12569	Country	US
Applicant 4			
Applicant Authority ☒ Inventor		☐ Legal Representative under 35 U.S.C. 117	☐ Party of Interest under 35 U.S.C. 118
Prefix	Given Name	Middle Name	Family Name
	Kelly		FRYE
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service			
City	Pomona	State/Province	NY
Country of Residence		US	
Citizenship under 37 CFR 1.41(b)		US	
Mailing Address of Applicant:			
Address 1	29 N. Ridge Road		
Address 2			
City	Pomona	State/Province	NY
Postal Code	10970	Country	US
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.			

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).			
☐ An Address is being provided for the correspondence information of this application.			
Customer Number	26693		
Email Address	joseph.zahner@regeneron.com	Add Email	Remove Email

Application Information:

Title of the Invention	VEGF Antagonist Formulations for Intravitreal Administration		
Attorney Docket Number	5050C	Small Entity Status Claimed ☐	
Application Type	Nonprovisional		
Subject Matter	Utility		
Suggested Class (if any)		Sub Class (if any)	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)		Suggested Figure for Publication (if any)	

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	5050C
		Application Number	
Title of Invention	VEGF Antagonist Formulations for Intravitreal Administration		

Publication Information:

☐	Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐	Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Enter either Customer Number or complete the Representative Name section below. If both sections are completed the Customer Number will be used for the Representative Information during processing.			
Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	26693		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.			
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	12560885	2009-09-16
Prior Application Status		Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12560885	Division of	11818463	2007-06-14
Prior Application Status		Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
11818463	non provisional of	60814484	2006-06-16
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			

Foreign Priority Information:

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(a).			
Remove			
Application Number	Country ¹	Parent Filing Date (YYYY-MM-DD)	Priority Claimed
			☐ Yes ☐ No

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	5050C
		Application Number	
Title of Invention	VEGF Antagonist Formulations for Intravitreal Administration		

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Assignee Information:

Providing this information in the application data sheet does not substitute for compliance with any requirement of part 3 of Title 37 of the CFR to have an assignment recorded in the Office.

Assignee 1

If the Assignee is an Organization check here. ☒

Organization Name Regeneron Pharmaceuticals, Inc.

Mailing Address Information:

Address 1 777 Old Saw Mill River Road

Address 2

City Tarrytown State/Province NY

Country US Postal Code 10591

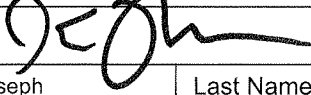
Phone Number 914-593-1595 Fax Number 914-345-7705

Email Address joseph.zahner@regeneron.com

Additional Assignee Data may be generated within this form by selecting the **Add** button.

Signature:

A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.

Signature			Date (YYYY-MM-DD)	2010-07-09	
First Name	Joseph	Last Name	Zahner	Registration Number	48224

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled

"VEGF ANTAGONIST FORMULATIONS SUITABLE FOR INTRAVITREAL ADMINISTRATION",
the specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to in the oath or declaration.

I acknowledge the duty to disclose information of which I am aware that is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

I hereby claim the benefit under Title 35, United States Code, §119(e) and 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States Application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) that occurred between the filing date of the prior application and the national or PCT international filing date of this application:

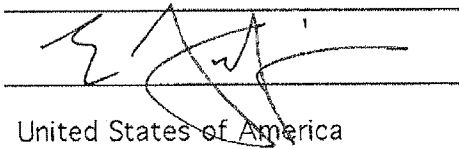
USSN 60/814,484, filed 16 June 2006

And I hereby appoint, Valeta Gregg (Registration No. 35,127), Tor Smeland (43,131), and Ying-Zi Yang (Registration No. 52,381), as my attorney and agent, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications that are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications, and direct all telephone calls, regarding this application to:

Valeta Gregg, Esq; Tor Smeland, Esq; and Ying-Zi Yang, Ph.D.
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, New York 10591
Tel. (914-345-7400)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Inventor: Eric FURFINE
Signature: 
Citizenship: United States of America
Residence: 315 Harrington Ave.
Concord, MA 01742
United States of America
Post Office Address: same as residence

Date: 25 Aug 2007

Inventor: Daniel DIX

Signature: _____

Date: _____

Citizenship: United States of America

Residence: 55 Memory Trail

LaGrangeville, New York 12540

United States of America

Post Office Address: same as residence

Inventor: Kenneth S. GRAHAM

Signature: Kenneth S. Graham

Date: 8/30/07

Citizenship: United States of America

Residence: 218 Robbout Road

Pleasant Valley, New York 12569

United States of America

Post Office Address: same as residence

Inventor: Kelly FRYE

Signature: Kelly Frye

Date: 8-17-07

Citizenship: United States of America

Residence: 29 N. Ridge Road

Pomona, New York 10970

United States of America

Post Office Address: same as residence

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled

“VEGF ANTAGONIST FORMULATIONS SUITABLE FOR INTRAVITREAL ADMINISTRATION”,
the specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to in the oath or declaration.

I acknowledge the duty to disclose information of which I am aware that is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

I hereby claim the benefit under Title 35, United States Code, §119(e) and 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States Application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) that occurred between the filing date of the prior application and the national or PCT international filing date of this application:

USSN 60/814,484, filed 16 June 2006

And I hereby appoint, Valeta Gregg (Registration No. 35,127), Tor Smeland (43,131), and Ying-Zi Yang (Registration No. 52,381), as my attorney and agent, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications that are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications, and direct all telephone calls, regarding this application to:

Valeta Gregg, Esq; Tor Smeland, Esq; and Ying-Zi Yang, Ph.D.
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, New York 10591
Tel. (914-345-7400)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Inventor: Eric FURFINE

Signature: _____

Date: _____

Citizenship: United States of America

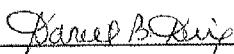
Residence: 315 Harrington Ave.

Concord, MA 01742

United States of America

Post Office Address: same as residence

Inventor: Daniel DIX

Signature: 

Date: 08-27-07

Citizenship: United States of America

Residence: 55 Memory Trail

LaGrangeville, New York 12540

United States of America

Post Office Address: same as residence

Inventor: Kenneth S. GRAHAM

Signature: _____

Date: _____

Citizenship: United States of America

Residence: 218 Robbout Road

Pleasant Valley, New York 12569

United States of America

Post Office Address: same as residence

Inventor: Kelly FRYE

Signature: _____

Date: _____

Citizenship: United States of America

Residence: 29 N. Ridge Road

Pomona, New York 10970

United States of America

Post Office Address: same as residence

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		TBA	
	Filing Date		2010-07-09	
	First Named Inventor	Eric FURFINE		
	Art Unit	TBA		
	Examiner Name	TBA		
	Attorney Docket Number	5050C		

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	6100071	A	2000-08-08	Davis-Smyth et al.	
	2	6897294	B2	2005-05-24	Davis-Smyth et al.	
	3	7052691	B2	2006-05-30	Sleeman et al.	

If you wish to add additional U.S. Patent citation information please click the Add button.

U.S.PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20060217311	A1	2006-09-28	DIX et al.	
	2	20050281831	A1	2005-12-22	Davis-Smyth et al.	

If you wish to add additional U.S. Published Application citation information please click the Add button.

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No	Foreign Document Number ³	Country Code ²	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T ⁵
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number			
	Filing Date		2010-07-09	
	First Named Inventor	Eric FURFINE		
	Art Unit	TBA		
	Examiner Name	TBA		
	Attorney Docket Number	5050C		

	1	WO 2006/047325			2006-05-04	Genentech, Inc.		☐
	2	WO 2005/000895			2005-01-06	Regeneron Phrma, Inc.		☐
	3	WO 2006/104852			2006-10-05	Regeneron Phrma, Inc.		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	1		☐

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	
Filing Date	2010-07-09
First Named Inventor	Eric FURFINE
Art Unit	TBA
Examiner Name	TBA
Attorney Docket Number	5050C

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ Fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ None

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature		Date (YYYY-MM-DD)	2010-07-09
Name/Print	Joseph Zahner	Registration Number	48,224

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
5 October 2006 (05.10.2006)

PCT

(10) International Publication Number
WO 2006/104852 A2

(51) International Patent Classification:

A61K 38/17 (2006.01) A61K 47/00 (2006.01)

(21) International Application Number:

PCT/US2006/010600

(22) International Filing Date: 22 March 2006 (22.03.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/665,125 25 March 2005 (25.03.2005) US

(71) Applicant (for all designated States except US): **REGENERON PHARMACEUTICALS, INC.** [US/US]; 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **DIX, Daniel** [US/US]; 55 Memory Trail, LaGrangeville, New York 12540 (US). **FRYE, Kelly** [US/US]; 29 N. Ridge Road, Pomona, New York 10970 (US). **KAUTZ, Susan** [US/US]; 3 Hall Place, Albany, New York 12210 (US).

(74) Agent: **VALETA, Gregg**; Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: VEGF ANTAGONIST FORMULATIONS

(57) Abstract: Formulations of a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist are provided including a pre-lyophilized formulation, a reconstituted lyophilized formulation, and a stable liquid formulation. Preferably, the fusion protein has the sequence of SEQ ID NO:4.

VEGF ANTAGONIST FORMULATIONS

Field of the Invention

[0001] The present invention is directed to pharmaceutical formulations comprising agents capable of inhibiting vascular endothelial growth factor (VEGF), and to methods for making and using such formulations. The invention includes pharmaceutical formulations having increased stability.

Statement of Related Art

[0002] Vascular endothelial growth factor (VEGF) expression is nearly ubiquitous in human cancer, consistent with its role as a key mediator of tumor neoangiogenesis. Blockade of VEGF function, by binding to the molecule or its VEGFR-2 receptor, inhibits growth of implanted tumor cells in multiple different xenograft models (see, for example, Gerber et al. (2000) Cancer Res. 60:6253-6258). A soluble VEGF-specific fusion protein antagonist, termed a "VEGF trap" has been described (Kim et al. (2002) Proc. Natl. Acad. Sci. USA 99:11399-404; Holash et al. (2002) Proc. Natl. Acad. Sci. USA 99:11393-8).

[0003] Lyophilization (freeze drying under controlled conditions) is commonly used for long term storage of proteins. The lyophilized protein is substantially resistant to degradation, aggregation, oxidation, and other degenerative processes while in the freeze-dried state (see, for example, U.S. 6,436,897).

BRIEF SUMMARY OF THE INVENTION

[0004] Stable formulations of a VEGF-specific fusion protein antagonist are herein provided. The pharmaceutically acceptable formulations of the invention comprise the VEGF "trap" antagonist with a pharmaceutically acceptable carrier. In specific embodiments, liquid and freeze-dried, or lyophilized formulations are provided.

[0005] In a first aspect, the invention features a stable liquid formulation of a VEGF-specific fusion protein antagonist, comprising a fusion protein comprising a receptor component consisting essentially of an immunoglobulin-like (Ig) domain 2 of a first VEGF receptor and Ig domain 3 of a second VEGF receptor, and a multimerizing component, one or more buffers, and one or more thermal stabilizers. In a specific embodiment of the VEGF-specific fusion protein antagonist, the first VEGF receptor is Flt1 and the second VEGF receptor is Flk1 or Flt4. In a more specific embodiment the fusion protein has the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. In one embodiment, the buffer is a phosphate buffer and/or citrate. More preferably, the buffers are phosphate and citrate. In one embodiment, the thermal stabilizers are NaCl and/or sucrose. More preferably, the thermal stabilizers are both NaCl and sucrose.

[0006] In a specific embodiment, the stable liquid formulation of a VEGF-specific fusion protein antagonist comprises 1-10 mM phosphate buffer, 1-10 mM citrate, 25-150 mM NaCl, 5-30%

sucrose, 10-50 mg/ml of the fusion protein, at a pH of about 6-6.5. In a more specific embodiment, the stable liquid formulation comprises 5 mM phosphate buffer, 5 mM citrate buffer, 100 mM NaCl, 20% sucrose, 25 mg/ml of the fusion protein, at a pH of about 6.0. Additionally, polysorbate may be present, for example 0.05-0.15% polysorbate 20. The stable liquid formulation of the VEGF-specific fusion protein antagonist of the invention exhibits little or no precipitation after storage of a 25 mg/ml VEGF formulation for about 6 months at -80°C and little or no precipitation after storage for 6 months at 5°C.

[0007] In a second aspect, the invention features a high concentration stable liquid formulation of a VEGF antagonist comprising 1-50 mM histidine, 25-150 mM NaCl, 5-30% sucrose, 50-100 mg/ml of the fusion protein, at a pH of about 6-6.5, and either 0.1-0.5% polysorbate or 1-5% PEG. In a more specific embodiment, the high concentration stable liquid formulation comprises 10 mM histidine, 50 mM NaCl, 5-20% sucrose, 50-100 mg/ml of the fusion protein, at a pH of about 6.0-6.5, with either 0.1% polysorbate (e.g., polysorbate 20) or 3% PEG (e.g., PEG 3350). The high concentration stable liquid formulation of the VEGF-specific fusion protein antagonist of the invention exhibits less than about 3% degradation after 15 months of storage at 5°C (75 or 100 mg/ml VEGF trap protein) or less than about 1.5% degradation after 24 months (50 mg/ml).

[0009] In a third aspect, the invention features a pre-lyophilized formulation of a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist, comprising a (i) fusion protein comprising a receptor component consisting essentially of an immunoglobulin-like (Ig) domain 2 of a first VEGF receptor and Ig domain 3 of a second VEGF receptor, and a multimerizing component, (ii) a buffer, (iii) an organic co-solvent or bulking agent, and (iv) one or more lyoprotectants. In various embodiments, the buffer is histidine, the organic co-solvent or bulking agent is PEG, and the lyoprotectant(s) is at least one of glycine and sucrose. In one embodiment, the pre-lyophilized formulation of the invention does not contain a preservative.

[0009] In one embodiment of the pre-lyophilized formulation of the invention, the formulation comprises 5-50 mM histidine, 0.1-3.0% PEG, 0.25-3.0% glycine, 0.5-6.0% sucrose, and 5-75 mg/ml of the fusion protein, at a pH of about 6.0- 6.5. In any embodiment, the pre-lyophilized formulation may further comprise up to 0.05 mM citrate and/or 0.003-0.005% polysorbate. The polysorbate present may be, for example, polysorbate 20.

[0010] In a more specific embodiment, the pre-lyophilized formulation comprises about 10 mM histidine, about 1.5% PEG 3350, about 0.75% glycine, about 2.5% sucrose, and about 12.5 to 75 mg/ml VEGF-specific fusion protein, at a pH of about 6.25. In specific embodiments, the fusion protein comprises the protein sequence of SEQ ID NO:4, present as a multimer, e.g., a dimer. In separate embodiments, the reconstituted formulation is 2 times the concentration of the pre-lyophilized formulation, e.g., a 20 mg fusion protein/ml pre-lyophilized formulation is reconstituted to a final formulation of 60 mg fusion protein/ml. Generally, the lyophilized

formulation is reconstituted with sterile water suitable for injection. In one embodiment, the reconstitution liquid may be bacteriostatic water.

[0011] In a preferred embodiment, the pre-lyophilized formulation consists essentially of about 10 mM histidine, about 1.5% PEG 3350, about 0.75% glycine, about 2.5% sucrose, and about 50 mg/ml of the fusion protein having the sequence of SEQ ID NO:4 as a dimer, at a pH of about 6.25. Citrate (less than or equal to about 0.02 mM) and/or polysorbate (less than or equal to about 0.0005%) may be present. Optionally, the pre-lyophilized formulation does not contain a preservative, a phosphate buffer, and/or more than trace amounts of NaCl. In one embodiment, the pre-lyophilized formulation consists of about 10 mM histidine, about 1.5% PEG 3350, about 0.75% glycine, about 2.5% sucrose, and about 50 mg/ml of the VEGF trap protein (SEQ ID NO:4), pH 6.3, and upon reconstitution contains 20 mM histidine, 3% PEG, 1.5% glycine, about 5% sucrose, and about 100 mg/ml VEGF trap protein.

[0012] In a fourth aspect, the invention features a method of producing a lyophilized formulation of a VEGF-specific fusion protein antagonist, comprising subjecting the pre-lyophilized formulation of the invention to lyophilization to generate a lyophilized formulation. The lyophilized formulation may be lyophilized by any method known in the art for lyophilizing a liquid.

[0013] In a fifth related aspect, the invention features a method of producing a reconstituted lyophilized formulation of a VEGF-specific fusion protein antagonist, comprising reconstituting the lyophilized formulation of the invention to a reconstituted formulation. In one embodiment, the reconstituted formulation is twice the concentration of the pre-lyophilized formulation, e.g., the method of the invention comprises: (a) producing a pre-lyophilized formulation of a VEGF-specific fusion protein antagonist, (b) subjecting the pre-lyophilized formulation of step (a) to lyophilization; and (c) reconstituting the lyophilized formulation of step (b).

[0014] In specific embodiments of the method of producing a reconstituted lyophilized formulation, a pre-lyophilized solution is present in a vial as a 25 mg VEGF-specific fusion protein antagonist per ml solution of pre-lyophilized formulation, which is lyophilized and reconstituted to an 50 mg/ml solution. In another embodiment, a 30 mg/ml pre-lyophilized solution is lyophilized and reconstituted to a 60 mg/ml solution. In another embodiment, a 40 mg/ml pre-lyophilized solution is lyophilized and reconstituted to a 80 mg/ml solution. In another embodiment, a 12.5 mg/ml pre-lyophilized solution is lyophilized and reconstituted to a 25 mg/ml solution. In another embodiment, a 50 mg/ml pre-lyophilized solution is lyophilized and reconstituted to a 100 mg/ml solution. In another embodiment, a 75 mg/ml pre-lyophilized solution is lyophilized and reconstituted to a 150 mg/ml solution. Preferably, the reconstituted lyophilized formulation does not contain a preservative.

[0015] Other objects and advantages will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION OF THE INVENTION

[0016] The present invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting unless indicated, since the scope of the present invention will be limited only by the appended claims.

[0017] As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Thus for example, references to “a method” include one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure.

General Description

[0018] Safe handling and administration of formulations comprising proteins represent significant challenges to pharmaceutical formulators. Proteins possess unique chemical and physical properties that present stability problems: a variety of degradation pathways exist for proteins, implicating both chemical and physical instability. Chemical instability includes deamination, aggregation, clipping of the peptide backbone, and oxidation of methionine residues. Physical instability encompasses many phenomena, including, for example, aggregation.

[0019] Chemical and physical stability can be promoted by removing water from the protein. Lyophilization (freeze-drying under controlled conditions) is commonly used for long-term storage of proteins. The lyophilized protein is substantially resistant to degradation, aggregation, oxidation, and other degenerative processes while in the freeze-dried state. The lyophilized protein is normally reconstituted with water optionally containing a bacteriostatic preservative (e.g., benzyl alcohol) prior to administration.

Definitions

[0020] The term “carrier” includes a diluent, adjuvant, excipient, or vehicle with which a composition is administered. Carriers can include sterile liquids, such as, for example, water and oils, including oils of petroleum, animal, vegetable or synthetic origin, such as, for example, peanut oil, soybean oil, mineral oil, sesame oil and the like.

[0021] The term “excipient” includes a non-therapeutic agent added to a pharmaceutical composition to provide a desired consistency or stabilizing effect. Suitable pharmaceutical excipients include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

[0022] The term “lyophilized” or “freeze-dried” includes a state of a substance that has been

subjected to a drying procedure such as lyophilization, where at least 50% of moisture has been removed.

[0023] The phrase "bulking agent" includes a compound that is pharmaceutically acceptable and that adds bulk to a lyo cake. Generally, acceptable bulking agents known to the art include, for example, carbohydrates, including simple sugars such as dextrose, ribose, fructose and the like, alcohol sugars such as mannitol, inositol and sorbitol, disaccharides including trehalose, sucrose and lactose, naturally occurring polymers such as starch, dextrans, chitosan, hyaluronate, proteins (e.g., gelatin and serum albumin), glycogen, and synthetic monomers and polymers. In the formulations of the invention, PEG 3350 is an organic co-solvent which is used to stabilize the fusion protein when agitated, mixed, or handled, and as a bulking agent to help produce an acceptable bulk.

[0024] The term "lyoprotectant" includes a substance that may be added to a freeze-dried or lyophilized formulation to help maintain protein structure when freeze-dried or lyophilized.

[0025] A "preservative" includes a bacteriostatic, bacteriocidal, fungistatic or fungicidal compound that is generally added to formulations to retard or eliminate growth of bacteria or other contaminating microorganisms in the formulations. Preservatives include, for example, benzyl alcohol, phenol, benzalkonium chloride, m-cresol, thimerosol, chlorobutanol, methylparaben, propylparaben and the like. Other examples of pharmaceutically acceptable preservatives can be found in the USP.

VEGF Antagonists

[0026] An VEGF antagonist is a compound capable of blocking or inhibiting the biological action of vascular endothelial growth factor (VEGF), and includes fusion proteins capable of trapping VEGF. In a preferred embodiment, the VEGF antagonist is the fusion protein of SEQ ID NO:2 or 4; more preferably, SEQ ID NO:4. In specific embodiments, the VEGF antagonist is expressed in a mammalian cell line such as a CHO cell and may be modified post-translationally. In a specific embodiment, the fusion protein comprises amino acids 27-457 of SEQ ID NO:4 and is glycosylated at Asn residues 62, 94, 149, 222 and 308.

[0027] The VEGF antagonist of the methods and formulations of the invention can be prepared by any suitable method known in the art, or that comes to be known. The VEGF antagonist is preferably substantially free of protein contaminants at the time it is used to prepare the pharmaceutically acceptable formulation. By "substantially free of protein contaminants" is meant, preferably, that at least 90 % of the weight of protein of the VEGF-specific fusion protein antagonist preparation used for making a formulation is VEGF fusion protein antagonist protein, more preferably at least 95%, most preferably at least 99%. The fusion protein is preferably substantially free of aggregates. "Substantially free of aggregates" means that at least 90% of the weight of fusion protein is not present in an aggregate at the time the fusion protein is used to prepare the pharmaceutically effective formulation. The fusion protein of the methods and

formulations of the invention may contain low or trace amounts of compounds as a results of the purification process, for example, low or trace amounts of citrate and/or polysorbate. In one embodiment of the pre-lyophilized formulation of the invention containing about 50 mg of fusion protein/ml, citrate may be present at a concentration of about 0.02 mM and/or polysorbate may be present at a concentration of about 0.0005%. If the pre-lyophilized formulation is reconstituted after lyophilization to half of the original volume (e.g., 100 mg/ml of fusion protein), the resulting concentrations may be 0.04 mM citrate and/or 0.001% polysorbate.

Lyophilization and Lyophilized Formulations

[0028] In one aspect of the invention, a pharmaceutically acceptable formulation comprising a VEGF-specific fusion protein antagonist is provided, wherein the formulation is a freeze-dried or lyophilized formulation. Lyophilized formulations can be reconstituted into solutions, suspensions, emulsions, or any other suitable form for administration or use. Lyophilized formulations are typically first prepared as liquids, then frozen and lyophilized. The total liquid volume before lyophilization can be less, equal to, or more than, the final reconstituted volume of the lyophilized formulation. The lyophilization process is well known to those of ordinary skill in the art, and typically includes sublimation of water from a frozen formulation under controlled conditions.

[0029] Lyophilized formulations can be stored at a wide range of temperatures. Lyophilized formulations may be stored below 25°C, for example, refrigerated at 4°C, or at room temperature (e.g., approximately 25°C). Preferably, lyophilized formulations are stored below about 25°C, more preferably, at about 4-20°C; below about 4°C; below about -20°C; about -40°C; about -70°C, or about -80°C.

[0030] Lyophilized formulations are typically reconstituted for use by addition of an aqueous solution to dissolve the lyophilized formulation. A wide variety of aqueous solutions can be used to reconstitute a lyophilized formulation. Preferably, lyophilized formulations are reconstituted using water. Lyophilized formulations are preferably reconstituted with a solution consisting essentially of water (e.g., USP WFI, or water for injection) or bacteriostatic water (e.g., USP WFI with 0.9% benzyl alcohol). However, solutions comprising buffers and/or excipients and/or one or more pharmaceutically acceptable carries can also be used.

[0031] Freeze-dried or lyophilized formulations are typically prepared from liquids, that is, from solutions, suspensions, emulsions, and the like. Thus, the liquid that is to undergo freeze-drying or lyophilization preferably comprises all components desired in a final reconstituted liquid formulation. As a result, when reconstituted, the freeze-dried or lyophilized formulation will render a desired liquid formulation upon reconstitution. A preferred liquid formulation used to generate a freeze-dried or lyophilized formulation comprises a VEGF-specific fusion protein antagonist in a pharmaceutically effective amount, a buffer, a stabilizer, and a bulking agent. Freeze-dried or lyophilized formulations preferably comprise histidine, since histidine, in

comparison to phosphate, is more effective at stabilizing the fusion protein when the fusion protein is lyophilized. Organic co-solvents, such as PEG 3350, are used to stabilize the fusion protein when agitated, mixed, or handled. A lyoprotectant is preferably used in freeze-dried or lyophilized formulations. Lyoprotectants help to maintain the secondary structure of proteins when freeze-dried or lyophilized. Two preferred example lyoprotectants are glycine and sucrose, which are preferably used together.

Stable Liquid Formulations

[0032] In one aspect, the invention provides a stable pharmaceutically acceptable formulation comprising a VEGF-specific fusion protein antagonist, wherein the formulation is a liquid formulation. Preferably, the liquid formulation comprises a pharmaceutically effective amount of the fusion protein. The formulation can also comprise one or more pharmaceutically acceptable carriers, buffers, bulking agents, stabilizers, preservatives, and/or excipients. An example of a pharmaceutically acceptable liquid formulation comprises a VEGF-specific fusion protein antagonist in a pharmaceutically effective amount, a buffer, a co-solvent, and one or more stabilizers.

[0033] A preferred liquid formulation comprises phosphate buffer, an organic co-solvent, and one or more thermal stabilizers to minimize formation of aggregates and low molecular weight products when stored, and about 10 mg/ml to about 50 mg/ml fusion protein, wherein the formulation is from about pH 6.0-6.5. A preferred liquid formulation comprises about 5 mM phosphate buffer, about 5 mM citrate, about 100 mM NaCl, about 25% sucrose, and about 10-50 mg/ml fusion protein, wherein the formulation is at a pH of about 6.0; optionally polysorbate may be present (e.g., 0.1% polysorbate 20). Although either NaCl or sucrose can be used as a stabilizer, a combination of NaCl and sucrose has been established to stabilize the fusion protein more effectively than either individual stabilizer alone.

[0034] Stability is determined in a number of ways at specified time points, including determination of pH, visual inspection of color and appearance, determination of total protein content by methods known in the art, e.g., UV spectroscopy, SDS-PAGE, size-exclusion HPLC, bioassay determination of activity, isoelectric focusing, and isoaspartate quantification. In one example of a bioassay useful for determining VEGF antagonist activity, a BAF/3 VEGFR1/EPOR cell line is used to determine VEGF₁₆₅ binding by the VEGF-specific fusion protein antagonist of the invention.

[0035] Formulations, whether liquid or freeze-dried and lyophilized, can be stored in an oxygen-deprived environment. Oxygen-deprived environments can be generated by storing the formulations under an inert gas such as, for example, argon, nitrogen, or helium.

EXAMPLES

[0036] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only to the appended claims.

[0037] As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0038] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

Example 1. Stability of a 50 mg/ml Liquid Formulation of VEGF Trap

[0039] A liquid formulation containing 10 mM phosphate, 50 mM NaCl, 0.1% polysorbate 20, 20% sucrose, and 50 mg/ml VEGF trap (SEQ ID NO:4), pH 6.25, was stored at 5 °C and samples tested at 3, 6, 9, 12, 18 and 24 months. Stability was determined by SE-HPLC. The results, shown in Table 1, show that 98.6% and 98.3% of VEGF trap protein remained intact (non-degraded) at 12 and 24 months, respectively. Turbidity was measured at OD₄₀₅ nm; and percent recovered protein by size exclusion HPLC.

Table 1. Stability of 50 mg/ml VEGF Trap Protein When Stored at 5 °C (VGFT-SS065)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.2	100	99.0
3	Pass	0.00	6.2	102	98.8
6	Pass	0.01	6.2	103	98.7
9	Pass	0.01	6.3	102	98.2
12	Pass	0.01	6.3	106	98.6
18	Pass	0.00	6.3	103	98.4
24	Pass	0.00	6.2	93	98.3

[0040] A liquid formulation containing 10 mM phosphate, 50 mM NaCl, 3% PEG 3350, 20%

sucrose, and 50 mg/ml VEGF trap (SEQ ID NO:4), pH 6.25, was stored at 5 °C and samples tested at 3, 6, 9, 12, 18 and 24 months. Stability results are shown in Table 2.

Table 2. Stability of 50 mg/ml VEGF Trap Protein When Stored at 5 °C (VGFT-SS065)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.2	100	99.0
3	Pass	0.00	6.2	100	98.8
6	Pass	0.01	6.3	103	98.5
9	Pass	0.00	6.3	103	98.3
12	Pass	0.01	6.3	110	98.3
18	Pass	0.00	6.3	113	98.0
24	Pass	0.01	6.2	90	97.8

Example 2. Stability of a 75 mg/ml Liquid Formulation of VEGF Trap

[0041] A liquid formulation containing 10 mM phosphate, 50 mM NaCl, 0.1% polysorbate 20, 20% sucrose, and 75 mg/ml VEGF trap (SEQ ID NO:4), pH 6.25, was stored at 5 °C and samples tested at 0, 1, 2.3, 3, 9, 12 and 15 months. Stability results are shown in Table 3.

Table 3. Stability of 75 mg/ml VEGF Trap Protein When Stored at 5 °C (VGFT-SS101)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.2	100	97.1
1	Pass	0.00	6.2	96	97.0
2.3	Pass	0.00	6.2	98	96.7
3	Pass	0.00	6.2	97	96.1
9	Pass	-0.01	6.0	101	96.0
12	Pass	0.00	6.3	110	94.5
15	Pass	0.00	6.3	92	95.6

[0042] A liquid formulation containing 10 mM phosphate, 50 mM NaCl, 3% PEG 3350, 20% sucrose, and 75 mg/ml VEGF trap (SEQ ID NO:4), pH 6.25, was stored at 5 °C and samples tested at 0, 1, 2.3, 3, 9, 12 and 15 months. Stability results are shown in Table 4.

Table 4. Stability of 75 mg/ml VEGF Trap Protein When Stored at 5 °C (VGFT-SS101)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.2	100	96.8
1	Pass	0.00	6.2	99	96.7
2.3	Pass	0.00	6.2	97	96.3
3	Pass	0.00	6.2	89	95.6
9	Pass	-0.01	6.2	98	95.4
12	Pass	-0.01	6.3	112	94.1
15	Pass	0.00	6.3	98	94.8

Example 3. Stability of a 100 mg/ml Liquid Formulation of VEGF Trap

[0043] A liquid formulation containing 10 mM phosphate, 50 mM NaCl, 0.1% polysorbate 20, 20% sucrose, and 100 mg/ml VEGF trap (SEQ ID NO:4), pH 6.25, was stored at 5 °C and samples tested at 0, 1, 2.3, 3, 9, 12 and 15 months. Stability results are shown in Table 5.

Table 5. Stability of 100 mg/ml VEGF Trap Protein Stored at 5 °C (VGFT-SS101)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.3	100	96.7
1	Pass	0.00	6.2	92	96.6
2.3	Pass	0.00	6.2	92	96.2
6	Pass	0.00	6.2	99	95.5
9	Pass	-0.01	6.2	92	95.5
12	Pass	-0.01	6.2	110	93.9
15	Pass	0.00	6.3	108	94.8

[0044] A liquid formulation containing 10 mM phosphate, 50 mM NaCl, 3% PEG 3350, 20% sucrose, and 100 mg/ml VEGF trap (SEQ ID NO:4), pH 6.25, was stored at 5 °C and samples tested at 0, 1, 2.3, 3, 9, 12 and 15 months. Stability results are shown in Table 6.

Table 6. Stability of 100 mg/ml VEGF Trap Protein Stored at 5 °C (VGFT-SS101)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.3	100	96.5
1	Pass	0.01	6.2	94	96.2
2.3	Pass	0.01	6.2	93	95.7
6	Pass	0.01	6.2	102	94.6
9	Pass	0.00	6.2	95	94.6
12	Pass	0.00	6.3	96	92.8
15	Pass	0.01	6.3	102	93.9

Example 4. Further Embodiments of Stable VEGF Trap Formulations

[0045] In one embodiment, the invention provides a stable liquid VEGF-binding fusion protein (VEGF trap) formulations comprising 5 mM phosphate, 5 mM citrate, 100 mM NaCl, 0.1% Polysorbate 20, 20% sucrose, 25 mg/ml VEGF trap protein, pH 6.0. This formulation can either be delivered subcutaneously or diluted and delivered by intravenous infusion. Due to the high osmolality of this formulation, it is diluted 3-fold to achieve an iso-osmolar solution for intravenous administration. Stability studies showed less than about 1 % degradation was detected after 3 years of storage at 2-8°C.

[0046] In one embodiment, the invention features a lyophilized formulation which is preferably concentrated two-fold from the pre-lyophilized to the post-lyophilized formulation, e.g., 50 to 100 mg/ml; 75 to 150 mg/ml, or 100 to 200 mg/ml VEGF trap protein. In one specific embodiment, the pre-lyophilized formulation comprises 10 mM histidine, 1.5% PEG 3350, 0.75% glycine, 2.5% sucrose, 50 mg/ml VEGF trap protein, pH 6.3, and is reconstituted to a formulation comprising 20 mM histidine, 3% PEG 3350, 1.5% glycine, 5% sucrose, 100 mg/ml VEGF trap protein, pH 6.3. Stability studied showed no degradation of the VEGF trap was detected after 6 months of storage at 2-8 °C.

[0047] In one embodiment of a liquid formulation, the formulation comprises 10 mM histidine, 50 mM NaCl, 5-20% sucrose, 50-100 mg/ml VEGF trap, and one of 0.1% polysorbate 20 or 3% PEG 3350. One advantage of this liquid formulation is that it provides a higher concentration of VEGF trap without requiring the manufacture of a lyophilized product. Thus, this formulation provides ease for subcutaneous delivery, for example, by allowing provision of a liquid pre-filled syringe at a concentration higher than that delivered by IV infusion. Also, this formulation could advantageously be used to provide lower infusion volumes and shorter infusion times. The amount of degradation determined by SE-HPLC following incubation at 5 °C for up to 15 or 24 months is summarized in Table 7.

Table 7. Stability of Liquid Formulation with 50-100 mg/ml VEGF Trap (VGFT-SS101)

Incubation (months)	VEGF Trap (mg/ml)	% Polysorbate 20	% PEG 3350	% Degradation
24	50	0.1	-	0.7
24	50	-	3	1.3
15	75	0.1	-	1.5
15	75	-	3	2.0
15	100	0.1	-	1.9
15	100	-	3	2.6

Example 5. Stability and Activity of Lyophilized and Liquid

[0048] The stability of a reconstituted lyophilized formulation was determined over a 6 month period. The pre-lyophilized formulation contained 10 mM histidine, 1.5% PEG 3350, 2.5% sucrose, 0.75% glycine and 50 mg/ml VEGF trap protein. After lyophilization, the reconstituted formulation contained 20 mM histidine, 3% PEG 3350, 5% sucrose, 1.5% glycine, and 100 mg/ml VEGF trap protein (SEQ ID NO:4). The results shown in Table 8. Activity was determined in a cell based bioassay which directly measures the ability of the VEGF trap to inhibit the biological effects of human VEGF on a mouse Baf/3 VEGFR1/EpoR cell line. Therefore, this bioassay directly measures the biological activity of the protein. The results are expressed as percent relative potency (test sample IC_{50} / reference VEGF IC_{50} standard x 100). The binding affinity of VEGF to the VEGF trap is measured using a sensitive ELISA that specifically measures free VEGF in equilibrated mixtures containing VEGF and various concentrations of the VEGF trap. Results are expressed as percent relative binding (IC_{50} of test sample/ IC_{50} of reference x 100). Measured pH ranged between 6.3 – 6.5. All solutions were visually clear. The concentration of VEGF trap recovered was determined with a UV spectrophotometer as mg/ml at A_{280} nm. The percent VEGF trap recovered in the native configuration (main peak purity) was determined with SE-HPLC.

Table 8. Stability of VEGF Trap Lyophilized Formulation Stored at 5 °C (VGT-RS475)

Months	Bioassay	Binding Assay	% Recovered	% Native Configuration
0	120	126	97.9	98.7
1	117	74	97.9	98.6
1 + 24 hr	126	72	99.0	98.5
1 + 4 hr	94	81	101.5	98.2
3	101	98	98.1	98.6
3 + 24 hr	65	94	98.1	98.2
6 + 4 hr			96.9	98.7
6 + 24 hr			98.8	98.6

[0049] A formulation containing about 5 mM phosphate, 5 mM citrate, 100 mM NaCl, 0.1% polysorbate 20, 20% sucrose, and 25 mg/ml VEGF trap protein was tested for stability and activity over 36 months when stored at 5 °C. The results are shown in Table 9. All samples were clear and colorless as determined by visual inspection. pH ranged from 6.0-6.1. *Binding assay results for two measurements (1 and 2 months) are expressed directly and not as a percent of the standard.

Table 9. Stability and Activity of Liquid Formulation (VGT-FS405)

Months	% Native Configuration	Bioassay	Binding Assay	Protein Content mg/ml
0	99.7	106	72	25.0
1	99.9	119	4.4 pM*	25.2
2	99.6	102	5.4 pM*	25.1
3	99.6	97	88	25.1
6	99.6	101	106	25.0
9	99.4	89	126	25.4
12	99.5	85	95	25.2
18	99.4	99	81	25.5
24	99.3	75	95	25.6
36	98.8	109	79	25.6

We claim:

1. A stable liquid formulation of a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist, comprising a fusion protein having a receptor component consisting of an immunoglobulin-like (Ig) domain 2 of a first VEGF receptor and Ig domain 3 of a second VEGF receptor, and a multimerizing component, one or more buffers, and one or more thermal stabilizers.
2. The stable liquid formulation of claim 1, wherein the first VEGF receptor is Flt1 and the second VEGF receptor is Flk1 or Flt4.
3. The stable liquid formulation of claim 2, wherein the fusion protein has the amino acid sequence of SEQ ID NO:4.
4. The stable liquid formulation of claim 1, wherein the buffer is at least one of phosphate buffer and citrate buffer.
5. The stable liquid formulation of claim 1, wherein the thermal stabilizer are at least one of NaCl and sucrose.
6. The stable liquid formulation of claim 1, comprising 1-10 mM phosphate buffer, 1-10 mM citrate, 25-150 mM NaCl, 5-30% sucrose, 10-50 mg/ml of the fusion protein, at a pH of about 6-6.5, optionally further comprising 0.05-0.10% polysorbate.
7. The stable liquid formulation of claim 6, comprising 5 mM phosphate buffer, 5 mM citrate buffer, 100 mM NaCl, 25% sucrose, 25 mg/ml of the fusion protein of SEQ ID NO:4, at a pH of about 6.0.
8. A pre-lyophilized formulation, comprising (i) a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist, (ii) a buffer, (iii) an organic co-solvent or bulking agent, and (iv) one or more lyoprotectants, wherein the fusion protein has the amino acid sequence of SEQ ID NO:4.
9. The pre-lyophilized formulation of claim 8, wherein the buffer is histidine.
10. The pre-lyophilized formulation of claim 8, wherein the organic co-solvent or bulking agent is PEG.

11. The pre-lyophilized formulation of claim 8, wherein the lyoprotectant(s) is at least one of glycine and sucrose.
12. The pre-lyophilized formulation of claim 8, comprising 5-50 mM histidine, 0.1-3.0% PEG, 0.25-3.0% glycine, 0.5-6.0% sucrose, and 5-75 mg/ml of the fusion protein, at a pH of about 6.0-6.5.
13. The pre-lyophilized formulation of claim 12, comprising about 10 mM histidine, about 1.5% PEG 3350, about 0.75% glycine, about 2.5% sucrose, and about 12.5 to 75 mg/ml VEGF-specific fusion protein, at a pH of about 6.25.
14. The pre-lyophilized formulation of claim 13, wherein the fusion protein is 50 mg/ml.
15. A liquid stable formulation of a VEGF antagonist, comprising 1-50 mM histidine, 25-150 mM NaCl, 5-30% sucrose, and 50-100 mg/ml of the fusion protein, at a pH of about 6.0- 6.5, optionally comprising 0.01-0.5% polysorbate, and optionally further comprising 0.1-5% PEG.
16. The liquid stable formulation of claim 15, comprising about 10 mM histidine, about 50 mM NaCl, 5-20% sucrose, and 50-100 mg/ml of the fusion protein, at a pH of about 6.0- 6.5, optionally comprising 0.1% polysorbate 20, and optionally further comprising 3% PEG 3350.
17. A method of producing a lyophilized formulation of a VEGF-specific fusion protein antagonist, comprising subjecting the pre-lyophilized formulation of claim 14 to lyophilization to generate a lyophilized formulation.
18. A method of producing a reconstituted lyophilized formulation of a VEGF-specific fusion protein antagonist, comprising reconstituting the lyophilized formulation claim 17 with liquid, wherein a reconstituted lyophilized formulation is generated.
19. The method of claim 18, wherein the liquid is sterile water or bacteriostatic water.

SEQUENCE LISTING

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<120> VEGF Antagonist Formulations

<130> 4030A-WO

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

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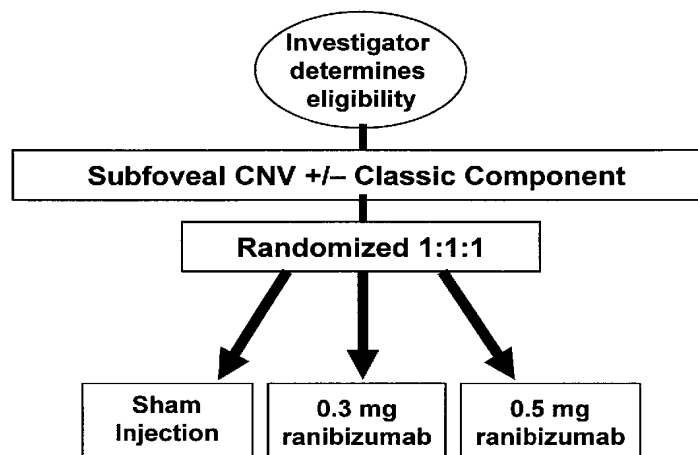
Published:

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(54) Title: METHOD FOR TREATING INTRAOCULAR NEOVASCULAR DISEASES

Trial Design



(57) Abstract: A method is provided for administering to a mammal suffering from, or at risk for, an intraocular neovascular disorder with regular dosing of a therapeutically effective amount of VEGF antagonist, followed by less frequent dosing of a therapeutically effective amount of VEGF antagonist.

METHOD FOR TREATING INTRAOCULAR NEOVASCULAR DISEASES

FIELD OF THE INVENTION

5 This invention relates to methods for treating an intraocular neovascular disorder with a VEGF antagonist. Methods for administering to a mammal suffering from, or at risk for, an intraocular neovascular disorder include monthly dosing of a therapeutically effective amount of VEGF antagonist, followed by less frequent dosing of a therapeutically effective amount of VEGF antagonist.

10

BACKGROUND OF THE INVENTION

Angiogenesis is implicated in the pathogenesis of intraocular neovascular diseases, e.g., proliferative retinopathies, age-related macular degeneration (AMD), etc., as well as a variety of other disorders. These include solid tumors, rheumatoid arthritis, and psoriasis (Folkman *et al. J. Biol. Chem.* 267:10931-10934 (1992); Klagsbrun *et al. Annu. Rev. Physiol.* 53:217-239 (1991); and Garner A, *Vascular diseases. In: Pathobiology of ocular disease. A dynamic approach.* Garner A, Klintworth GK, Eds. 2nd Edition Marcel Dekker, NY, pp 1625-1710 (1994)).

20 The search for positive regulators of angiogenesis has yielded many candidates, including aFGF, bFGF, TGF- α , TGF- β HGF, TNF- α , angiogenin, IL-8, etc. (Folkman *et al.* and Klagsbrun *et al.*). The negative regulators so far identified include thrombospondin (Good *et al. Proc. Natl. Acad. Sci. USA.* 87:6624-6628 (1990)), the 16-kilodalton N-terminal fragment of prolactin (Clapp *et al. Endocrinology*, 133:1292-1299 (1993)), angiostatin (O'Reilly *et al. Cell*, 79:315-328 (1994)) and endostatin (O'Reilly *et al. Cell*, 88:277-285 (1996)).

30 Work done over the last several years has established the key role of vascular endothelial growth factor (VEGF) in the regulation of normal and abnormal angiogenesis (Ferrara *et al. Endocr. Rev.* 18:4-25 (1997)). The finding that the loss of even a single VEGF allele results in embryonic lethality points to an irreplaceable role played by this factor in the development and differentiation of the vascular system (Ferrara *et al.*).

Human VEGF exists as at least six isoforms (VEGF₁₂₁, VEGF₁₄₅, VEGF₁₆₅, VEGF₁₈₃, VEGF₁₈₉, and VEGF₂₀₆) that arise from alternative splicing of mRNA of a single gene (Ferrara N, Davis Smyth T. *Endocr Rev* 18:1–22 (1997)). VEGF₁₆₅, the most abundant isoform, is a basic, heparin binding, dimeric glycoprotein with a molecular mass of ~45,000 daltons (*Id*).

5 Two VEGF receptor tyrosine kinases, VEGFR1 and VEGFR2, have been identified (Shibuya et al. *Oncogene* 5:519–24 (1990); Matthews et al., *Proc Natl Acad Sci U S A* 88:9026–30 (1991); Terman et al., *Oncogene* 6:1677–83 (1991); Terman et al. *Biochem Biophys Res Commun* 187:1579–86 (1992); de Vries et al., *Science* 255:989–91 (1992); Millauer et al. *Cell* 72:835–46 (1993); and, Quinn et al. *Proc Natl Acad Sci USA* 90:7533–7 (1993)). VEGFR1
10 has the highest affinity for VEGF, with a K_d of ~10–20 pM (de Vries et al., *Science* 255:989–91 (1992)), and VEGFR2 has a somewhat lower affinity for VEGF, with a K_d of ~75–125 pM (Terman et al., *Oncogene* 6:1677–83 (1991); Millauer et al. *Cell* 72:835–46 (1993); and, Quinn et al. *Proc Natl Acad Sci USA* 90:7533–7 (1993)).

15 VEGF has several biologic functions, including regulation of VEGF gene expression under hypoxic conditions (Ferrara N, Davis Smyth T. *Endocr Rev* 18:1–22 (1997)), mitogenic activity for micro and macrovascular endothelial cells (Ferrara N, Henzel WJ. *Biochem Biophys Res Commun* 161:851–8 (1989); Leung et al., *Science* 246:1306–9 (1989); Connolly et al. *J Clin Invest* 84:1470–8 (1989a); Keck et al. *Science* 246:1309–12 (1989); Plouet et al.,
20 *EMBO J* 8:3801–6 (1989); Conn et al. *Proc Natl Acad Sci USA* 87:2628–32 (1990); and, Pepper et al., *Exp Cell Res* 210:298–305 (1994)), and induction of expression of plasminogen activators and collagenase (Pepper et al., *Biochem Biophys Res Commun* 181:902–6 (1991)).

Furthermore, VEGF has been shown to be a key mediator of neovascularization associated
25 with tumors and intraocular disorders (Ferrara *et al.*). The VEGF mRNA is overexpressed by the majority of human tumors examined. Berkman *et al.* *J Clin Invest* 91:153–159 (1993); Brown *et al.* *Human Pathol* 26:86–91 (1995); Brown *et al.* *Cancer Res* 53:4727–4735 (1993); Mattern *et al.* *Brit J Cancer*. 73:931–934 (1996); and Dvorak *et al.* *Am J Pathol* 146:1029–1039 (1995). Also, the concentration of VEGF in eye fluids are highly correlated to the
30 presence of active proliferation of blood vessels in patients with diabetic and other ischemia-related retinopathies. Aiello *et al.*, *N. Engl. J. Med.* 331:1480–1487 (1994). Furthermore, recent studies have demonstrated the localization of VEGF in choroidal neovascular membranes in patients affected by AMD. Lopez *et al.*, *Invest. Ophthalmol. Vis. Sci.* 37:855–868 (1996); Kvant *et al.*, *Invest Ophthalmol Vis Sci* 37:1929–34 (1996).

Age related macular degeneration (AMD) is a leading cause of severe, irreversible vision loss among the elderly. Bressler, *JAMA* 291:1900-1 (2004). It is characterized by a broad spectrum of clinical and pathologic findings, such as pale yellow spots known as drusen, disruption of the retinal pigment epithelium (RPE), choroidal neovascularization (CNV), and disciform macular degeneration. The manifestations of the disease are classified into two forms: non exudative (dry) and exudative (wet or neovascular). Drusen are the characteristic lesions of the dry form, and neovascularization characterizes the wet form. Disciform AMD is the fibrotic stage of the neovascular lesion.

There is a dramatic increase in the prevalence of AMD with advancing age. *See, e.g.,* Leibowitz et al., *Surv Ophthalmol* 24(Suppl):335-610 (1980) and Klein et al., *Ophthalmology* 99:933-43 (1992). Although the wet form of AMD is much less common, it is responsible for 80%-90% of the severe visual loss associated with AMD (Ferris et al., *Arch Ophthalmol* 102:1640-2 (1984)). There is an estimated 1-1.2 million prevalent cases of wet AMD. The cause of AMD is unknown; however, it is clear that the risk of developing AMD increases with advancing age. Other known risk factors include family history and cigarette smoking. Postulated risk factors also include oxidative stress, diabetes, alcohol intake, and sunlight exposure. D'Amico, *N Engl J Med* 331:95-106 (1994) and Christen et al., *JAMA* 276:1147-51 (1996).

Dry AMD is characterized by changes in the RPE and Bruch's membrane. It is thought that the RPE, compromised by age and other risk factors, deposits lipofuscin and cellular debris on Bruch's membrane. These changes may be seen ophthalmoscopically as drusen, which are scattered throughout the macula and posterior retinal pole. There are also variable degrees of atrophy and pigmentation of the RPE. Dry AMD may be asymptomatic or accompanied by variable and usually minimal visual loss and is considered to be a prelude to development of wet AMD.

Wet AMD is typically characterized by CNV of the macular region. The choroidal capillaries proliferate and penetrate Bruch's membrane to reach the RPE and may extend into the subretinal space. The increased permeability of the newly formed capillaries leads to accumulation of serous fluid or blood under the RPE and/or the neurosensory retina or within the neurosensory retina. When the fovea becomes swollen or detached, decreases in vision

occur. Fibrous metaplasia and organization may ensue, resulting in an elevated subretinal mass called a disciform scar that constitutes end-stage AMD and is associated with permanent vision loss (D'Amico DJ. *N Engl J Med* 331:95-106 (1994)).

5 The neovascularization in AMD can be classified into different patterns based on fluorescein angiography of subfoveal choroidal neovascular lesions. TAP and VIP Study Groups, *Arch Ophthalmol* 121:1253-68 (2003). The major angiographic patterns are termed classic and occult and are associated with different degrees of aggressiveness, vision losses, and response to different treatment options.

10 The diffusible nature of VEGF and its specificity of action for endothelial cells support a key role in the process of abnormal blood vessel growth and vascular leakage. Increased expression of VEGF in retinal photoreceptors or RPE of transgenic mice stimulates neovascularization within the retina, and VEGF antagonists partially inhibit retinal
15 neovascularization in animal models (Okamoto et al. *Am J Pathol* 151:281-91 (1997); Schwesinger et al., *AM J Pathol.* Mar;158(3):1161-72 (2001)). Anti-VEGF neutralizing antibodies inhibit intraocular angiogenesis in models of ischemic retinal disorders (Adamis et al. *Arch. Ophthalmol.* 114:66-71 (1996)), and also suppress the growth of a variety of human tumor cell lines in nude mice (Kim et al. *Nature* 362:841-844 (1993); Warren et al. *J. Clin.*
20 *Invest.* 95:1789-1797 (1995); Borgström et al. *Cancer Res.* 56:4032-4039 (1996); and Melnyk et al. *Cancer Res.* 56:921-924 (1996)). Therefore, anti-VEGF monoclonal antibodies or other VEGF antagonists are promising candidates for use in treatments of intraocular neovascular disorders, and new methods of administering therapeutic compounds, which increases the effectiveness of the therapeutic compound, are needed.

25 SUMMARY OF THE INVENTION

One object of the present invention is to provide an improved method of administering a therapeutic compound. This and other objects will become apparent from the following description.

30 Methods for treating intraocular neovascular disease are provided. For example, methods include administering to a mammal a number of first individual doses of a VEGF antagonist, followed by administering to the mammal a number of second individual doses of the

antagonist, wherein the second individual doses are administered less frequently than the first individual doses.

In one embodiment of the invention, a method for treating wet form age-related macular degeneration is provided, which comprises administering to a mammal a number of first individual doses of an VEGF antagonist, followed by administering to the mammal a number of second individual doses of the antagonist, wherein the second individual doses are administered less frequently than the first individual doses.

10 In one embodiment, the mammal is in need of treatment. Typically, the mammal is a human.

In one embodiment, the administration of the VEGF antagonist is ocular. In one aspect, the administration is intraocular. In another aspect, the administration is intravitreal.

15 A VEGF antagonist is administered in the methods of the invention. In one aspect, the VEGF antagonist is an anti-VEGF antibody, e.g., a full length anti-VEGF antibody or an antibody fragment. In one embodiment, the anti-VEGF antibody is a Fab antibody fragment. In one embodiment, the antibody fragment is Y0317.

20 In one embodiment of the invention, the first individual doses are administered at one month intervals (e.g., about 3 individual doses). Typically, there is more than one first individual dose. In another embodiment, the second individual doses are administered at three month intervals (e.g., about 6 individual doses). In one aspect of the invention, the second individual doses are administered beginning three months after the number of first individual doses. In
25 one embodiment, a number of second individual doses are administered to the mammal during a period of at least 22 months following the number of first individual doses.

In one embodiment of the invention, the number of first individual doses and the number of second individual doses are administered over a time period of about 2 years. In one aspect,
30 the first individual dose is administered at month 0, 1 and 2. In another aspect, the second individual dose is administered at month 5, 8, 11, 14, 17, 20 and 23. For example, the first individual dose is administered at month 0, 1, and 2 and the second individual dose is administered at month 5, 8, 11, 14, 17, 20 and 23. In one embodiment, the VEGF antagonist is administered over less than 2 years, or optionally, administered over greater than 2 years.

Other aspects of the invention will become apparent from the following description of the embodiments which are not intended to be limiting of the invention.

5

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 schematically illustrates the study in Example 1.

Figure 2 schematically illustrates a dosing regimen for treating, e.g., age-related macular degeneration (AMD) with a VEGF antagonist.

10

DETAILED DESCRIPTION

Definitions

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

20

The term "human VEGF" as used herein refers to the 165-amino acid human vascular endothelial cell growth factor, and related 121-, 189-, and 206-, (and other isoforms) amino acid vascular endothelial cell growth factors, as described by Leung *et al.*, *Science* 246:1306 (1989), and Houck *et al.*, *Mol. Endocrin.* 5:1806 (1991) together with the naturally occurring allelic and processed forms of those growth factors.

25

A "VEGF antagonist" refers to a molecule capable of neutralizing, blocking, inhibiting, abrogating, reducing or interfering with VEGF activities including its binding to one or more VEGF receptors. VEGF antagonists include anti-VEGF antibodies and antigen-binding fragments thereof, receptor molecules and derivatives which bind specifically to VEGF thereby sequestering its binding to one or more receptors, anti-VEGF receptor antibodies and VEGF receptor antagonists such as small molecule inhibitors of the VEGFR tyrosine kinases, and fusions proteins, e.g., VEGF-Trap (Regeneron), VEGF₁₂₁-gelonin (Peregrine). VEGF antagonists also include antagonist variants of VEGF, antisense molecules directed to VEGF,

RNA aptamers specific to VEGF, and ribozymes against VEGF or VEGF receptors.

Antagonists of VEGF act by interfering with the binding of VEGF to a cellular receptor, by incapacitating or killing cells which have been activated by VEGF, or by interfering with vascular endothelial cell activation after VEGF binding to a cellular receptor. All such points

5 of intervention by a VEGF antagonist shall be considered equivalent for purposes of this invention. Preferred VEGF antagonists are anti-VEGF antagonistic antibodies capable of inhibiting one or more of the biological activities of VEGF, for example, its mitogenic, angiogenic or vascular permeability activity. Anti-VEGF antagonistic antibodies include, but not limited to, antibodies A4.6.1, rhuMab VEGF (bevacizumab), Y0317 (ranibizumab), G6,
10 B20, 2C3, and others as described in, for example, WO98/45331, US2003/0190317, U.S. Patents 6,582,959 and 6,703,020; WO98/45332; WO 96/30046; WO94/10202; WO2005/044853; EP 0666868B1; and Popkov et al., *Journal of Immunological Methods* 288:149-164 (2004). More preferably, the anti-VEGF antagonistic antibody of the invention is ranibizumab, which is a humanized, affinity matured anti-human VEGF antibody Fab
15 fragment having the light and heavy chain variable domain sequences of Y0317 as described in WO98/45331 and Chen et al *J Mol Biol* 293:865-881 (1999).

The antibody is appropriately from any source, including chicken and mammalian such as rodent, goat, primate, and human. Typically, the antibody is from the same species as the
20 species to be treated, and more preferably the antibody is human or humanized and the host is human. While the antibody can be a polyclonal or monoclonal antibody, typically it is a monoclonal antibody, which can be prepared by conventional technology. The antibody is an IgG-1, -2, -3, or -4, IgE, IgA, IgM, IgD, or an intraclass chimera in which Fv or a CDR from one class is substituted into another class. The antibody may have an Fc domain capable of an
25 effector function or may not be capable of binding complement or participating in ADCC.

The term "VEGF receptor" or "VEGFr" as used herein refers to a cellular receptor for VEGF, ordinarily a cell-surface receptor found on vascular endothelial cells, as well as variants thereof which retain the ability to bind hVEGF. One example of a VEGF receptor is the *fms*-
30 like tyrosine kinase (*flt*), a transmembrane receptor in the tyrosine kinase family. DeVries et al., *Science* 255:989 (1992); Shibuya et al., *Oncogene* 5:519 (1990). The *flt* receptor comprises an extracellular domain, a transmembrane domain, and an intracellular domain with tyrosine kinase activity. The extracellular domain is involved in the binding of VEGF, whereas the intracellular domain is involved in signal transduction. Another example of a

VEGF receptor is the *flk-1* receptor (also referred to as KDR). Matthews *et al.*, *Proc. Nat. Acad. Sci.* 88:9026 (1991); Terman *et al.*, *Oncogene* 6:1677 (1991); Terman *et al.*, *Biochem. Biophys. Res. Commun.* 187:1579 (1992). Binding of VEGF to the *flt* receptor results in the formation of at least two high molecular weight complexes, having apparent molecular weight of 205,000 and 300,000 Daltons. The 300,000 Dalton complex is believed to be a dimer comprising two receptor molecules bound to a single molecule of VEGF.

The term "epitope A4.6.1" when used herein, unless indicated otherwise, refers to the region of human VEGF to which the A4.6.1 antibody disclosed in Kim *et al.*, *Growth Factors* 7:53 (1992) and Kim *et al.* *Nature* 362:841 (1993), binds.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, *etc.* Typically, the mammal is human.

The term "antibody" is used in the broadest sense and includes monoclonal antibodies (including full length or intact monoclonal antibodies), polyclonal antibodies, multivalent antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments (see below) so long as they exhibit the desired biological activity.

Unless indicated otherwise, the expression "multivalent antibody" is used throughout this specification to denote an antibody comprising three or more antigen binding sites. The multivalent antibody is typically engineered to have the three or more antigen binding sites and is generally not a native sequence IgM or IgA antibody.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges.

Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework region (FR). The variable domains of native heavy and light chains each comprise four FRs (FR1, FR2, FR3 and FR4, respectively), largely adopting a β -sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991), pages 647-669). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity (ADCC).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*i.e.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.*

196:901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H - V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteine(s) from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain

constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

- 5 "Antibody fragments" comprise only a portion of an intact antibody, generally including an antigen binding site of the intact antibody and thus retaining the ability to bind antigen. Examples of antibody fragments encompassed by the present definition include: (i) the Fab fragment, having VL, CL, VH and CH1 domains; (ii) the Fab' fragment, which is a Fab fragment having one or more cysteine residues at the C-terminus of the CH1 domain; (iii) the
- 10 Fd fragment having VH and CH1 domains; (iv) the Fd' fragment having VH and CH1 domains and one or more cysteine residues at the C-terminus of the CH1 domain; (v) the Fv fragment having the VL and VH domains of a single arm of an antibody; (vi) the dAb fragment (Ward et al., *Nature* 341, 544-546 (1989)) which consists of a VH domain; (vii) isolated CDR regions; (viii) F(ab')₂ fragments, a bivalent fragment including two Fab' fragments linked by a disulphide bridge at the hinge region; (ix) single chain antibody
- 15 molecules (e.g. single chain Fv; scFv) (Bird et al., *Science* 242:423-426 (1988); and Huston et al., *PNAS (USA)* 85:5879-5883 (1988)); (x) "diabodies" with two antigen binding sites, comprising a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (see, e.g., EP 404,097; WO 93/11161; and Hollinger et al.,
- 20 *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); (xi) "linear antibodies" comprising a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata et al. *Protein Eng.* 8(10):1057-1062 (1995); and US Patent No. 5,641,870).
- 25 The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody
- 30 preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the

monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature* 256:495 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described
5 in Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or
10 homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567;
15 and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)).

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which
20 hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in
25 the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized
30 antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Reichmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al. *Nature Biotechnology* 14:309-314 (1996); Sheets et al. *PNAS (USA)* 95:6157-6162 (1998)); Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991)). Human antibodies can also be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., *Bio/Technology* 10: 779-783 (1992); Lonberg et al., *Nature* 368: 856-859 (1994); Morrison, *Nature* 368:812-13 (1994); Fishwild et al., *Nature Biotechnology* 14: 845-51 (1996); Neuberger, *Nature Biotechnology* 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995). Alternatively, the human antibody may be prepared via immortalization of human B lymphocytes producing an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or may have been immunized *in vitro*). See, e.g., Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner et al., *J. Immunol.*, 147 (1):86-95 (1991); and US Pat No. 5,750,373.

The term “Fc region” is used to define the C-terminal region of an immunoglobulin heavy chain which may be generated by papain digestion of an intact antibody. The Fc region may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at about position Cys226, or from about position Pro230, to the carboxyl-terminus of the Fc region. The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain.

By “Fc region chain” herein is meant one of the two polypeptide chains of an Fc region.

The "CH2 domain" of a human IgG Fc region (also referred to as "Cg2" domain) usually extends from an amino acid residue at about position 231 to an amino acid residue at about position 340. The CH2 domain is unique in that it is not closely paired with another domain.

5 Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It has been speculated that the carbohydrate may provide a substitute for the domain-domain pairing and help stabilize the CH2 domain. Burton, *Molec. Immunol.*22:161-206 (1985). The CH2 domain herein may be a native sequence CH2 domain or variant CH2 domain.

10 The "CH3 domain" comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (i.e. from an amino acid residue at about position 341 to an amino acid residue at about position 447 of an IgG). The CH3 region herein may be a native sequence CH3 domain or a variant CH3 domain (e.g. a CH3 domain with an introduced "protroberance" in one chain
15 thereof and a corresponding introduced "cavity" in the other chain thereof; see US Patent No. 5,821,333, expressly incorporated herein by reference). Such variant CH3 domains may be used to make multispecific (e.g. bispecific) antibodies as herein described.

"Hinge region" is generally defined as stretching from about Glu216, or about Cys226, to
20 about Pro230 of human IgG1 (Burton, *Molec. Immunol.*22:161-206 (1985)). Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S-S bonds in the same positions. The hinge region herein may be a native sequence hinge region or a variant hinge region. The two polypeptide chains of a variant hinge region generally retain at least one cysteine residue per
25 polypeptide chain, so that the two polypeptide chains of the variant hinge region can form a disulfide bond between the two chains. The preferred hinge region herein is a native sequence human hinge region, e.g. a native sequence human IgG1 hinge region.

A "functional Fc region" possesses at least one "effector function" of a native sequence Fc
30 region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding

domain (e.g. an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

5 A “native sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature.

10 A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will typically possess, e.g., at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, or at least about 90% sequence identity therewith, or at least about 95% sequence or more identity therewith.

20 “Antibody-dependent cell-mediated cytotoxicity” and “ADCC” refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in US Patent No. 5,500,362 or 25 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. *PNAS (USA)* 95:652-656 (1998).

30 “Human effector cells” are leukocytes which express one or more FcRs and perform effector functions. Typically, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with

PBMCs and NK cells being generally preferred. The effector cells may be isolated from a native source thereof, e.g. from blood or PBMCs as described herein.

The terms "Fc receptor" and "FcR" are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (reviewed in Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-92 (1991); Capel et al., *Immunomethods* 4:25-34 (1994); and de Haas et al., *J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976); and Kim et al., *J. Immunol.* 24:249 (1994)).

"Complement dependent cytotoxicity" and "CDC" refer to the lysing of a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996), may be performed.

An "affinity matured" antibody is one with one or more alterations in one or more CDRs thereof which result an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al. *Bio/Technology* 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas et

al. *Proc Nat. Acad. Sci, USA* 91:3809-3813 (1994); Schier et al. *Gene* 169:147-155 (1995); Yelton et al. *J. Immunol.* 155:1994-2004 (1995); Jackson et al., *J. Immunol.* 154(7):3310-9 (1995); and Hawkins et al, *J. Mol. Biol.* 226:889-896 (1992).

5 A "flexible linker" herein refers to a peptide comprising two or more amino acid residues joined by peptide bond(s), and provides more rotational freedom for two polypeptides (such as two Fd regions) linked thereby. Such rotational freedom allows two or more antigen binding sites joined by the flexible linker to each access target antigen(s) more efficiently. Examples of suitable flexible linker peptide sequences include gly-ser, gly-ser-gly-ser, ala-ser, and gly-
10 gly-gly-ser.

"Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which
15 enables the sFv to form the desired structure for antigen binding. For a review of sFv see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which
20 fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*,
25 *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993).

The expression "linear antibodies" when used throughout this application refers to the antibodies described in Zapata *et al. Protein Eng.* 8(10):1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (V_H-C_H1-V_H-C_H1) which form a pair of
30 antigen binding regions. Linear antibodies can be bispecific or monospecific.

A "variant" anti-VEGF antibody, refers herein to a molecule which differs in amino acid sequence from a "parent" anti-VEGF antibody amino acid sequence by virtue of addition,

deletion and/or substitution of one or more amino acid residue(s) in the parent antibody sequence. In the preferred embodiment, the variant comprises one or more amino acid substitution(s) in one or more hypervariable region(s) of the parent antibody. For example, the variant may comprise at least one, *e.g.* from about one to about ten, and preferably from about two to about five, substitutions in one or more hypervariable regions of the parent antibody. Ordinarily, the variant will have an amino acid sequence having at least 75% amino acid sequence identity with the parent antibody heavy or light chain variable domain sequences, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the parent antibody residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody sequence shall be construed as affecting sequence identity or homology. The variant retains the ability to bind human VEGF and preferably has properties which are superior to those of the parent antibody. For example, the variant may have a stronger binding affinity, enhanced ability to inhibit VEGF-induced proliferation of endothelial cells and/or increased ability to inhibit VEGF-induced angiogenesis *in vivo*. To analyze such properties, one should compare a Fab form of the variant to a Fab form of the parent antibody or a full length form of the variant to a full length form of the parent antibody, for example, since it has been found that the format of the anti-VEGF antibody impacts its activity in the biological activity assays disclosed, *e.g.*, in WO98/45331 and US2003/0190317. In one embodiment, the variant antibody is one which displays at least about 10 fold, preferably at least about 20 fold, and most preferably at least about 50 fold, enhancement in biological activity when compared to the parent antibody.

The "parent" antibody herein is one which is encoded by an amino acid sequence used for the preparation of the variant. Preferably, the parent antibody has a human framework region and, if present, has human antibody constant region(s). For example, the parent antibody may be a humanized or human antibody.

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In

preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The term "epitope tagged" when used herein refers to the anti-VEGF antibody fused to an "epitope tag." The epitope tag polypeptide has enough residues to provide an epitope against which an antibody thereagainst can be made, yet is short enough such that it does not interfere with activity of the VEGF antibody. The epitope tag preferably is sufficiently unique so that the antibody thereagainst does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least 6 amino acid residues and usually between about 8-50 amino acid residues (preferably between about 9-30 residues). Examples include the flu HA tag polypeptide and its antibody 12CA5 (Field *et al. Mol. Cell. Biol.* 8:2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan *et al., Mol. Cell. Biol.* 5(12):3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky *et al., Protein Engineering* 3(6):547-553 (1990)). In certain embodiments, the epitope tag is a "salvage receptor binding epitope". As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG₁, IgG₂, IgG₃, or IgG₄) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

An "angiogenic factor or agent" is a growth factor which stimulates the development of blood vessels, *e.g.*, promotes angiogenesis, endothelial cell growth, stability of blood vessels, and/or vasculogenesis, etc. For example, angiogenic factors, include, but are not limited to, *e.g.*, VEGF and members of the VEGF family, PlGF, PDGF family, fibroblast growth factor family (FGFs), TIE ligands (Angiopoietins), ephrins, ANGPTL3, ANGPTL4, etc. It would also include factors that accelerate wound healing, such as growth hormone, insulin-like growth factor-I (IGF-I), VIGF, epidermal growth factor (EGF), CTGF and members of its family, and TGF- α and TGF- β . See, *e.g.*, Klagsbrun and D'Amore, *Annu. Rev. Physiol.*, 53:217-39

(1991); Streit and Detmar, *Oncogene*, 22:3172-3179 (2003); Ferrara & Alitalo, *Nature Medicine* 5(12):1359-1364 (1999); Tonini et al., *Oncogene*, 22:6549-6556 (2003) (e.g., Table 1 listing angiogenic factors); and, Sato *Int. J. Clin. Oncol.*, 8:200-206 (2003).

- 5 An "anti-angiogenesis agent" or "angiogenesis inhibitor" refers to a small molecular weight substance, a polynucleotide, a polypeptide, an isolated protein, a recombinant protein, an antibody, or conjugates or fusion proteins thereof, that inhibits angiogenesis, vasculogenesis, or undesirable vascular permeability, either directly or indirectly. For example, an anti-angiogenesis agent is an antibody or other antagonist to an angiogenic agent as defined above,
- 10 e.g., antibodies to VEGF, antibodies to VEGF receptors, small molecules that block VEGF receptor signaling (e.g., PTK787/ZK2284, SU6668). Anti-angiogenesis agents also include native angiogenesis inhibitors, e.g., angiostatin, endostatin, etc. *See, e.g.*, Klagsbrun and D'Amore, *Annu. Rev. Physiol.*, 53:217-39 (1991); Streit and Detmar, *Oncogene*, 22:3172-3179 (2003) (e.g., Table 3 listing anti-angiogenic therapy in malignant melanoma); Ferrara &
- 15 Alitalo, *Nature Medicine* 5(12):1359-1364 (1999); Tonini et al., *Oncogene*, 22:6549-6556 (2003) (e.g., Table 2 listing antiangiogenic factors); and, Sato *Int. J. Clin. Oncol.*, 8:200-206 (2003) (e.g., Table 1 lists Anti-angiogenic agents used in clinical trials).

The term "effective amount" or "therapeutically effective amount" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of age-related macular degeneration (AMD), the effective amount of the drug can reduce or prevent vision loss. For AMD therapy, efficacy in vivo can, for example, be measured by one or more of the following: assessing the mean change in the best corrected visual acuity (BCVA) from baseline to a desired time, assessing the proportion of subjects who lose fewer than 15 letters

20 in visual acuity at a desired time compared with baseline, assessing the proportion of subjects who gain greater than or equal to 15 letters in visual acuity at a desired time compared with baseline, assessing the proportion of subjects with a visual-acuity Snellen equivalent of 20/2000 or worse at desired time, assessing the NEI Visual Functioning Questionnaire, assessing the size of CNV and amount of leakage of CNV at a desired time , as assessed by

25 fluorescein angiography, etc.

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A therapeutic dose is a dose which exhibits a therapeutic effect on the patient and a sub-therapeutic dose is a dose which does not exhibit a therapeutic effect on the patient treated.

An "intraocular neovascular disease" is a disease characterized by ocular neovascularization. Examples of intraocular neovascular diseases include, but are not limited to, e.g., proliferative retinopathies, choroidal neovascularization (CNV), age-related macular degeneration (AMD), diabetic and other ischemia-related retinopathies, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, retinal neovascularization, etc.

The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody. The label may itself be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g. controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g. an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as the anti-VEGF antibodies) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

MODES OF THE INVENTION

It has been discovered that the treatment effects of a VEGF antagonist, e.g., Ranibizumab, are maintained for an extended period of time, such as more than one month. Treatment with the VEGF antagonist was also found to be well tolerated for up to 2 years. The present invention describes a treatment schedule comprising an initial interval of administration of a therapeutic compound, followed by a subsequent, less frequent interval of administration of the therapeutic compound. The methods of the present invention allow one to decrease subsequent doses of the therapeutic compound, while at the same time maintaining the therapeutic efficacy.

The therapeutic compound which is administered using the treatment schedule of the present invention is a VEGF antagonist, preferably an anti-VEGF antibody (e.g., Ranibizumab).

VEGF is a secreted homodimeric protein that is a potent vascular endothelial cells mitogen (Ferrara N, Davis Smyth T. *Endocr Rev* 18:1–22 (1997)). VEGF stimulates vascular endothelial cell growth, functions as a survival factor for newly formed vessels, and induces vascular permeability. VEGF expression is upregulated by hypoxia as well as by a number of other stimuli.

In methods of the invention, therapeutic effects of a VEGF antagonist are provided by administering to a mammal a number of first individual doses of an VEGF antagonist; followed by, administering to the mammal a number of second individual doses of the antagonist, where the second individual doses are administered less frequently than the first individual doses.

The term "therapeutic" in this context means that the compounds binds to the ligand, VEGF, and produce a change in the symptoms or conditions associated with the disease or condition which is being treated. It is sufficient that a therapeutic dose produce an incremental change in the symptoms or conditions associated with the disease; a cure or complete remission of symptoms is not required. One having ordinary skill in this art can easily determine whether a dose is therapeutic by establishing criteria for measuring changes in symptoms or conditions of the disease being treated and then monitoring changes in these criteria according to known methods. External physical conditions, histologic examination of affected tissues in patients or the presence or absence of specific cells or compounds, associated with a disease may provide objective criteria for evaluating therapeutic effect. In one example, methods of the invention may be used to treat AMD where therapeutic effect is assessed by changes in preventing vision loss. Other indicators of therapeutic effect will be readily apparent to one having ordinary skill in the art and may be used to establish efficacy of the dose. See also section entitled herein, "Efficacy of the Treatment."

The doses may be administered according to any time schedule which is appropriate for treatment of the disease or condition. For example, the dosages may be administered on a daily, weekly, biweekly or monthly basis in order to achieve the desired therapeutic effect and reduction in adverse effects. The dosages can be administered before, during or after the development of the disorder. The specific time schedule can be readily determined by a

physician having ordinary skill in administering the therapeutic compound by routine adjustments of the dosing schedule within the method of the present invention. The time of administration of the number of first individual and second individual doses as well as subsequent dosages is adjusted to minimize adverse effects while maintaining a maximum therapeutic effect. The occurrence of adverse effects can be monitored by routine patient interviews and adjusted to minimize the occurrence of side effects by adjusting the time of the dosing. Any dosing time is to be considered to be within the scope of the present invention so long as the number of first individual doses of the VEGF antagonist is administered followed by a number of second individual doses, which are less frequently administered. For example, doses may be administered on a monthly schedule followed by subsequent quarterly or more dose schedule. Maintenance doses are also contemplated by the invention.

In a further embodiment, the first individual dose may be repeated one or more times before the second individual dose is administered. The first dose may be administered, for example, one, two or three times, typically three times before the less frequent administration dose(s) is (are) administered. In one embodiment of the invention, the first individual doses are administered at one month intervals (e.g., about 3 individual doses). The second dose is administered less frequently, e.g., at three month intervals (e.g., about 6 individual doses). In one aspect of the invention, the second individual doses are administered beginning three months after the number of first individual doses.

In one embodiment of the invention, the number of first individual doses and the number of second individual doses are administered over a time period of about 2 years. Shorter and longer time periods of 2 years are also included in the invention. In one aspect, the first individual dose is administered at month 0, 1 and 2. In another aspect, the second individual dose is administered at month 5, 8, 11, 14, 17, 20 and 23. In one example, the first individual dose is administered at month 0, 1, and 2 and the second individual dose is administered at month 5, 8, 11, 14, 17, 20 and 23.

Another aspect of the invention is the treatment of an intraocular neovascular disease, e.g., wet form AMD, by administering to a mammal, preferably a human patient, a number of first individual doses of a compound, e.g., a VEGF antagonist, followed by administering a number of second individual doses of the compound, where the number of second individual doses are administered less frequently than the number of first individual doses. This aspect of the

invention is different than previous dosing methods for the treatment of such diseases which generally treat with regularly spaced, even doses of a therapeutic compound. For example, the Ranibizumab (rhuFab V2), which is an antihuman VEGF, affinity-matured Fab has been administered in equal monthly (about 28 days) doses of 0.3 mg or 0.5 mg. In contrast, the method of the invention provides a number of first individual doses which are typically evenly spaced follow by a number of second individual doses that are less frequently administered.

The patient receives an initial dose of the VEGF antagonist. Since the VEGF antagonist treatment effects are maintained for more than a month, the patient can receive less frequent doses of the therapeutic compound in subsequent doses. However, it is possible to give more frequent doses, within the scope of the invention, to patients who do not experience effects on first administration.

The dosage amount depends on the specific disease or condition which is treated and can be readily determined using known dosage adjustment techniques by a physician having ordinary skill in treatment of the disease or condition. The dosage amount will generally lie with an established therapeutic window for the therapeutic compound which will provide a therapeutic effect while minimizing additional morbidity and mortality. Typically, therapeutic compounds are administered in a dosage ranging from 0.001 mg to about 100 mg per dose, preferably 0.1-20 mg.

Also within the scope of the present invention are additional doses, which may be administered after the number of first individual doses and after the number of second individual doses. For example, an additional, third set of doses can be administered.

Typically, the therapeutic compound used in the methods of this invention is formulated by mixing it at ambient temperature at the appropriate pH, and at the desired degree of purity, with physiologically acceptable carriers, i.e., carriers that are non-toxic to recipients at the dosages and concentrations employed. The pH of the formulation depends mainly on the particular use and the concentration of antagonist, but preferably ranges anywhere from about 3 to about 8. Where the therapeutic compound is an anti-VEGF antibody (e.g., ranibizumab), a suitable embodiment is a formulation at about pH 5.5.

The therapeutic compound, e.g. an anti-VEGF antibody, for use herein is preferably sterile. Sterility can be readily accomplished by sterile filtration through (0.2 micron) membranes. Preferably, therapeutic peptides and proteins are stored as aqueous solutions, although lyophilized formulations for reconstitution are acceptable.

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The therapeutic compound may be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the time scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of the therapeutic compound to be administered is governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat an intraocular neovascular disease.

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The therapeutic compound for treatment of an intraocular neovascular disease is typically administered by ocular, intraocular, and/or intravitreal injection. Other methods administration by also be used, which includes but is not limited to, topical, parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, and intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. As described herein, the therapeutic compound for treatment of an intraocular neovascular syndrome may be formulated, dosed, and administered in a fashion consistent with good medical practice.

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The efficacy of the treatment of the invention can be measured by various endpoints commonly used in evaluating intraocular neovascular diseases. For example, vision loss can be assessed. Vision loss can be evaluated by, but not limited to, e.g., measuring by the mean change in best correction visual acuity (BCVA) from baseline to a desired time point (e.g., where the BCVA is based on Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart and assessment at a test distance of 4 meters), measuring the proportion of subjects who lose fewer than 15 letters in visual acuity at a desired time point compared to baseline, measuring the proportion of subjects who gain greater than or equal to 15 letters in visual acuity at a desired time point compared to baseline, measuring the proportion of subjects with a visual-acuity Snellen equivalent of 20/2000 or worse at a desired time point, measuring the NEI Visual Functioning Questionnaire, measuring the size of CNV and amount

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of leakage of CNV at a desired time point, e.g., by fluorescein angiography, etc. Ocular assessments can be done, e.g., which include, but are not limited to, e.g., performing eye exam, measuring intraocular pressure, assessing visual acuity, measuring slitlamp pressure, assessing intraocular inflammation, etc.

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Any compound which binds to VEGF or a VEGF receptor and reduces the severity of symptoms or conditions associated with an intraocular neovascular disease may be used in this embodiment of the invention. Preferred compounds are peptide or protein compounds, more preferably are compounds which are or which contain an antibody or fragment thereof or which are fusions to an antibody fragment such as an immunoadhesin. Particularly preferred compounds are anti-VEGF antibodies or compounds containing fragments thereof.

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VEGF is expressed in a variety of cells in the normal human retina. Co-localization of VEGF mRNA and protein is observed in the ganglion cell, inner nuclear and outer plexiform layers, the walls of the blood vessels, and photoreceptors (Gerhardinger et al., *Am J Pathol* 152:1453–62 (1998)). Retinal pigment epithelium, Muller cells, pericytes, vascular endothelium, and ganglion cells all produce VEGF (Miller et al., *Diabetes Metab Rev* 13:37–50 (1997); and, Kim et al. *Invest Ophthalmol Vis Sci* 40:2115–21 (1999)).

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Studies have documented the immunohistochemical localization of VEGF in surgically resected CNV membranes from AMD patients. Kvant et al. (1996) demonstrated the presence of VEGF mRNA and protein in RPE cells and fibroblast like cells. See Kvant et al., *Invest Ophthalmol Vis Sci* 37:1929–34 (1996). Lopez et al. (1996) noted that the RPE cells that were strongly immunoreactive for VEGF were present primarily in the highly vascularized regions of CNV membranes, whereas the RPE cells found in fibrotic regions of CNV membranes showed little VEGF reactivity. See Lopez et al., *Invest Ophthalmol Vis Sci* 37:855–68 (1996). Kliffen et al. (1997) also demonstrated increased VEGF expression in RPE cells and choroidal blood vessels in maculae from patients with wet AMD compared with controls. See Kliffen et al., *Br J Ophthalmol* 81:154–62 (1997).

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An increase in VEGF expression has been noted in experimental models of CNV in rats and in non human primates (Husain et al., *Ophthalmology* 104:1242–50 (1997); and, Yi et al. Vascular endothelial growth factor expression in choroidal neovascularization in rats. *Graefes Arch Clin Exp Ophthalmol* 235:313–9 (1997)). In addition, transgenic mice with increased VEGF

expression in photoreceptors (Okamoto et al. 1997, *supra*) or retinal pigment epithelium (Schwesinger et al., *AM J Pathol.* 158(3):1161-72 (2001)) developed neovascularization reminiscent of CNV seen in humans with neovascular AMD. This further supports the involvement of VEGF in ocular neovascularization.

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Of particular relevance to wet AMD are the angiogenic properties of VEGF, which have been demonstrated in a variety of in vivo models, including the chick chorioallantoic membrane (Leung et al., *Science* 246:1306-9 (1989); and, Plouet J, Schilling J, Gospodarowicz D. *EMBO J* 8:3801-6 (1989)), rabbit cornea (Phillips et al., *In Vivo* 8:961-5 (1994)), and rabbit bone (Connolly et al. *J Clin Invest* 84:1470-8 (1989a)). VEGF also functions as a survival factor for newly formed endothelial cells (Dvorak HF. *N Engl J Med* 315:1650-9 (1986); and, Connolly et al. *J Biol Chem* 264:20017-24 (1989b)). Consistent with pro survival activity, VEGF induces expression of the anti apoptotic proteins Bcl 2 and A1 in human endothelial cells (Connolly et al. *J Biol Chem* 264:20017-24 (1989b)).

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VEGF has been shown to induce vascular leakage in guinea pig skin (Connolly et al. *J Biol Chem* 264:20017-24 (1989b)). Dvorak (1986) and colleagues (1987) proposed that an increase in microvascular permeability is a crucial step in angiogenesis associated with tumors and wound healing. Dvorak HF. *N Engl J Med* 315:1650-9 (1986); and, Dvorak et al., *Lab Invest* 57:673-86 (1987). A major function of VEGF in the angiogenic process can be the induction of plasma protein leakage. This effect would result in the formation of an extravascular fibrin gel, which serves as a substrate for endothelial cells. This activity can have relevance for AMD, as it is well established that permeability of the CNV membranes results in transudation of serum components beneath and into the retina, leading to serous macular detachment, macular edema and vision loss.

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Thus, VEGF antagonists are good therapeutic compounds for treating intraocular neovascular diseases.

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Many therapeutic compounds are well known to exert a therapeutic effect by binding to a selective cell surface marker or receptor or ligand. These known therapeutic compounds, e.g., anti-angiogenesis agents, are apparent to one having ordinary skill in the art and may be used in the method of the present invention. Suitable therapeutic compounds include non-peptidic organic compounds, preferably having a molecular weight less than about 1,000 g/mol, more

preferably less than about 600 g/mol; peptide therapeutic compounds, generally containing 8 to about 200, preferably about 15 to about 150, more preferably about 20 to about 100 amino acid residues; and protein therapeutic compounds, generally having secondary, tertiary and possibly quaternary structure. Suitable peptide compounds can be prepared by known solid-phase synthesis or recombinant DNA technology which are well known in the art.

A particularly preferred method of selecting a peptide compound is through the use of phage display technology. Using known phage display methods, libraries of peptides or proteins are prepared in which one or more copies of individual peptides or proteins are displayed on the surface of a bacteriophage particle. DNA encoding the particular peptide or protein is within the phage particle. The surface-displayed peptides or proteins are available for interaction and binding to target molecules which are generally immobilized on a solid support such as a 96-well plate or chromatography column support material. Binding and/or interaction of the display peptide or protein with a target molecule under selected screening conditions allows one to select members of the library which bind or react with the target molecule under the selected conditions. For example, peptides which bind under particular pH or ionic conditions may be selected. Alternatively, a target cell population can be immobilized on a solid surface using known techniques and the peptide or protein phage library can be panned against the immobilized cells to select peptides or proteins which bind to cell surface receptors on the target cell population. Phage display techniques are disclosed, for example, in U.S. Pat. Nos. 5,750,373; 5,821,047; 5,780,279; 5,403,484; 5,223,407; 5,571,698; and others.

One category of polypeptide compounds, are compounds containing an antibody or a fragment thereof which immunologically recognize and bind to cell surface receptors or ligands.

Methods of preparing antibodies are well known in the art and have been practiced for many years. Suitable antibodies may be prepared using conventional hybridoma technology or by recombinant DNA methods. Preferred antibodies are humanized forms of non-human antibodies. Alternatively, antibodies may be prepared from antibody phage libraries using methods described, for example, in U.S. Pat. Nos. 5,565,332; 5,837,242; 5,858,657; 5,871,907; 5,872,215; 5,733,743, and others. Suitable compounds include full-length antibodies as well as antibody fragments such as Fv, Fab, Fab' and F(ab')₂ fragments which can be prepared by reformatting the full length antibodies using known methods.

Additional preferred polypeptide therapeutic compounds are immunoadhesin molecules also known as hybrid immunoglobulins. These polypeptides are useful as cell adhesion molecules and ligands and also useful in therapeutic or diagnostic compositions and methods. An immunoadhesin typically contains an amino acid sequence of a ligand binding partner protein fused at its C-terminus to the N-terminus of an immunoglobulin constant region sequence. Immunoadhesins and methods of preparing the same are described in U.S. Pat. Nos. 5,428,130; 5,714,147; 4,428,130; 5,225,538; 5,116,964; 5,098,833; 5,336,603; 5,565,335; etc.

Pharmaceutical Compositions

Therapeutic compounds of the invention used in accordance with the present invention are prepared for storage by mixing a polypeptide(s) having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEENTM, PLURONICSTM or polyethylene glycol (PEG).

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in

macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. In one embodiment of the invention, an intraocular implant can be used for providing the VEGF antagonist. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing a polypeptide of the invention, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

EXAMPLES

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: Dosing Regimen

This study assesses the efficacy and safety of intravitreal injections of VEGF antagonist (e.g., ranibizumab) administered monthly for 3 doses followed by doses every 3 months compared with sham injections administered at the same schedule in subjects with primary or recurrent subfoveal choroidal neovascularization (CNV) with or without a classic CNV component secondary to AMD.

In this study, two treatment groups receive multiple intravitreal doses of VEGF antagonist from 0.3 mg to 0.5 mg for 24 months. See **Figure 1**. Each dose of VEGF antagonist is administered every month for 3 doses (Day 0, Month 1 and Month 2) followed by doses every 3 months (Months 5, 8, 11, 14, 17, 20, and 23) until study termination. See **Figure 2**. Subjects randomized to sham injections follow the same schedule as subjects receiving ranibizumab. During the 24 month study period, a total of 10 ranibizumab or 10 sham injections can be administered. Typically, the dosing does not occur earlier than 14 days after the previous treatment. If a dose is withheld or is missed, it may be optionally administered within 14 days following the previous treatment during the monthly injection period or within 45 days after the previous treatment during the 3-month dosing period. A maximum of 10 doses of study drug is administered during this study. Ranibizumab is administered in one eye only (study eye) during this study.

An example of a VEGF antagonist is ranibizumab (LUCENTISTM). Ranibizumab (rhuFab V2) is a humanized, affinity-matured anti-human VEGF Fab fragment. Ranibizumab is produced by standard recombinant technology methods in Escherichia coli expression vector and bacterial fermentation. Ranibizumab is not glycosylated and has a molecular mass of ~48,000 daltons. See WO98/45331 and US20030190317.

Ranibizumab Injection: For intravitreal administration, the study drug, ranibizumab, is supplied in a liquid-filled vial of ranibizumab. Each vial contains 0.7 mL of either 6 mg/mL (0.3 mg dose level) or 10 mg/mL (0.5-mg dose level) of ranibizumab aqueous solution (pH 5.5) with 10 mM of histidine, 100 mg/mL of trehalose, and 0.01% polysorbate 20. All study drug is stored at 2°C–8°C (36°F–46°F), and should not be frozen. Drug should be protected vials from direct sunlight.

Procedures are implemented to minimize the risk of potential adverse events associated with serial intraocular injections (e.g., endophthalmitis). Aseptic technique is observed for the injection tray assembly, anesthetic preparation and administration, and study drug preparation and administration.

5

Intravitreal injections are performed by the injecting physician(s) following the slitlamp examination. After thorough cleansings of the lid, lashes, and periorbital area with an antiseptic, local anesthesia and antimicrobials can administered prior to study drug injection.

- 10 A 30 gauge, 1/2-inch needle attached to a low volume (e.g., tuberculin) syringe containing 50 μ L of study drug solution is inserted through the pre anesthetized conjunctiva and sclera, approximately 3.5–4.0 mm posterior to the limbus, avoiding the horizontal meridian and aiming toward the center of the globe. The injection volume should be delivered slowly. The needle is then be removed slowly to ensure that all drug solution is in the eye. Immediately
- 15 following the intraocular injection, antimicrobial drops can be administered and the subject is instructed to self-administer antimicrobial drops four times daily for 3 days following each intraocular injection of ranibizumab. The scleral site for subsequent intravitreal injections should be rotated.
- 20 *Sham Injection:* The injecting physician(s) performs the same pre-injection cleansing and anesthetizing procedures (including subconjunctival injection of anesthesia) outlined above for subjects receiving ranibizumab. An empty syringe without a needle is used in the sham injection. The injecting physician(s) mimics an intraocular injection by making contact with the conjunctiva and applying pressure without the needle. Immediately following the sham
- 25 injection, the injecting physician(s) performs the same post-injection procedures as those performed on subjects receiving ranibizumab.

- Pre-Injection Procedures for All Subjects (Raninizumab or Sham Injection):* The following procedures can be implemented to minimize the risk of potential adverse events associated
- 30 with serial intravitreal injections (e.g., endophthalmitis). Aseptic technique is observed for injection tray assembly, anesthetic preparation, and study drug preparation and administration. The following procedures (except where noted) can be conducted by the physician performing the intravitreal injection of ranibizumab or sham injection. Subjects receive antimicrobials

(e.g., ofloxacin ophthalmic solution or trimethoprim polymyxin B ophthalmic solution) for self-administration four times daily for 3 days prior to treatment.

- The supplies are assembled and a sterile field is prepared. Supplies can include 10% povidone iodine swabs, sterile surgical gloves, 4X4 sterile pads, pack of sterile cotton tipped applicators, eyelid speculum, sterile ophthalmic drape, 0.5% proparacaine hydrochloride, 5% povidone iodine ophthalmic solution, 1% lidocaine for injection, ophthalmic antimicrobial solution (e.g., ofloxacin ophthalmic solution or trimethoprim polymyxin B ophthalmic solution, single-use vial), and injection supplies.
- 2 drops of 0.5% proparacaine hydrochloride are instilled into the study eye, followed by 2 drops of a broad spectrum antimicrobial solution (e.g., ofloxacin ophthalmic solution or trimethoprim polymyxin B ophthalmic solution, single-use vial).
- The periocular skin and eyelid of the study eye are disinfected in preparation for injection. The eyelid, lashes, and periorbital skin are scrubbed with 10% povidone iodine swabs, starting with the eyelid and lashes and continuing with the surrounding periocular skin. The eyelid margins and lashes are swabbed, e.g., in a systematic fashion, from medial to temporal aspects.
- A sterile ophthalmic drape can be placed to isolate the field, and the speculum can be placed underneath the eyelid of the study eye.
- 2 drops of 5% povidone iodine ophthalmic solution are instilled in the study eye, making sure the drops cover the planned injection site on the conjunctiva.
- Wait 90 seconds.
- A sterile cotton-tipped applicator is saturated with 0.5% proparacaine hydrochloride drops and the swab is held against the planned intravitreal injection site for 10 seconds in preparation for the subconjunctival injection of 1% lidocaine hydrochloride ophthalmic solution for injection (without epinephrine).
- 1% lidocaine (without epinephrine) is injected subconjunctivally.
- A sterile 4X4 pad in a single wipe can be used to absorb excess liquid and to dry the periocular skin.
- The subject is instructed to direct gaze away from syringe prior to ranibizumab or sham injection.

Ranibizumab Preparation and Administration Instructions: The ranibizumab injection can be prepared as herein. Dose solutions are typically prepared immediately before dosing. Dose solutions are typically for single use only.

After preparing the study eye as outlined above, 0.2 mL ranibizumab dose solution is withdrawn through a 5- μ m filter needle. The filter needle is removed and replaced with a 30-gauge, ½ inch Precision Glide® needle, and excess ranibizumab is expelled so that the syringe contains 0.05 mL ranibizumab solution. The syringe is inserted through an area 3.5–4.0 mm posterior to the limbus, avoiding the horizontal meridian and aiming toward the center of the globe. The injection volume should be delivered slowly. The needle is then removed slowly to ensure about all drug solution is in the eye. The scleral site for subsequent intravitreal injections should be rotated. Refer to next section for detailed post injection procedures.

The subject can be monitored with a finger count test for the study eye within, e.g., 15 minutes of the ranibizumab injection. A measurement of intraocular pressure of the study eye can be obtained, e.g., 60 minutes ($\pm$ 10 minutes) following the ranibizumab injection.

Post-Injection Procedures for All Subjects: Immediately following the ranibizumab or sham injection, 2 drops of antimicrobial drops (e.g., ofloxacin ophthalmic solution or trimethoprim polymyxin B ophthalmic solution, single-use vial) are instilled in the study eye. The subject is instructed to self-administer antimicrobial drops (e.g., ofloxacin ophthalmic solution or trimethoprim polymyxin B ophthalmic solution, single-use vial) four times daily for 3 days following each injection (ranibizumab or sham).

Preparation and Administration of the Sham Injection: See above for detailed instructions for pre-injection procedures.

Subjects receiving sham injections do not receive an actual injection of study drug. The physician follows the procedures for cleansing and anesthetizing the study eye as outlined above. The subject should be instructed to direct his or her gaze away from the syringe prior to administration of the sham injection. The tuberculin syringe plunger is withdrawn to the 0.05 mL mark on the syringe, the hub of the syringe—without the needle—is then placed against the pre-anesthetized conjunctival surface. The syringe hub is pressed firmly against the globe and then the plunger is slowly depressed, mimicking the action of an intravitreal injection.

For subsequent sham injections, the procedure of rotating the location of the injection site, as is done with ranibizumab injections is followed. See above for detailed post-injection procedures.

- 5 The subject can be monitored using a finger count test within, e.g., 15 minutes of the sham injection. A measurement of intraocular pressure can be obtained, e.g., 60 minutes (± 10 minutes) following the sham injection.

- 10 Safety is assessed by the incidence of ocular and non-ocular adverse events, including but not limited to, serious adverse events, ocular assessments, deaths, laboratory test results, vital signs, antibodies to Ranibizumab, intraocular inflammation, visual acuity, intraocular pressure, slitlamp pressure, indirect ophthalmoscopy, fluorescein angiography, fundus photography, vitreous hemorrhage, sensory rhegmatogenous retinal break or detachment (including macular hole), subfoveal hemorrhage, local or systemic infection, intraocular surgery, etc. In one embodiment, if verteporfin PDT was given within the last 28 days, the ranibizumab/sham injection is withheld. Efficacy is assessed by changes in preventing vision loss, e.g., measured by the mean change in best correction visual acuity (BCVA) from baseline to 12 months or 24 months (where the BCVA is based on the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart and assessment at a test distance of 4 meters), other means include but are not limited to measuring the proportion of subjects who lose fewer than 15 letters in visual acuity at 12 months or 24 months compared to baseline, measuring the proportion of subjects who gain greater than or equal to 15 letters in visual acuity at 12 months or 24 months compared to baseline, measuring the proportion of subjects with a visual-acuity Snellen equivalent of 20/2000 or worse at 12 months or 24 months, measuring the NEI Visual Functioning Questionnaire, measuring the size of CNV and amount of leakage of CNV at 12 months or 24 months, e.g., by fluorescein angiography.

- 30 The specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

CLAIMS

We claim:

1. A method for treating wet form age-related macular degeneration in a mammal, comprising
5 the steps of:
 - a) administering to the mammal a number of first individual doses of an VEGF antagonist; and
 - b) administering to the mammal a number of second individual doses of the VEGF antagonist, wherein the second individual doses are administered less frequently than the first individual doses.
- 10 2. The method of claim 1, wherein the mammal is a human.
3. The method of claim 1, wherein the administration is ocular.
- 15 4. The method of claim 3, wherein the administration is intraocular.
5. The method of claim 4, wherein the administration is intravitreal.
6. The method of claim 1, wherein the VEGF antagonist is an anti-VEGF antibody.
- 20 7. The method of claim 6, wherein the anti-VEGF antibody is a full length anti-VEGF antibody.
8. The method of claim 6, wherein the anti-VEGF antibody is an antibody fragment.
- 25 9. The method of claim 6, wherein the anti-VEGF antibody is a Fab antibody fragment.
10. The method of claim 8, wherein the antibody fragment is Y0317.
- 30 11. The method of claim 1, wherein the first individual doses are administered at one month intervals.
12. The method of claim 1, wherein the second individual doses are administered at three month intervals.

13. The method of claim 1, wherein the second individual doses are administered beginning three months after the number of first individual doses.

5 14. The method of claim 1, wherein the number of second individual doses are administered to the mammal during a period of at least 22 months following the number of first individual doses.

10 15. The method of claim 1, wherein the number of the first individual doses comprises about 3 individual doses.

16. The method of claim 1, wherein the number of the second individual doses comprises about 6 individual doses.

15 17. The method of claim 1, wherein the number of first individual doses and the number of second individual doses are administered over a time period of about 2 years.

20 18. The method of claim 1, wherein the first individual dose is administered at month 0, 1 and 2.

19. The method of claim 1, wherein the second individual dose is administered at month 5, 8, 11, 14, 17, 20 and 23.

25 20. The method of claim 1, wherein the first individual dose is administered at month 0, 1, and 2 and the second individual dose is administered at month 5, 8, 11, 14, 17, 20 and 23.

21. A method for treating intraocular neovascular disease, comprising:
administering to a mammal a number of first individual doses of an VEGF antagonist;
followed by,

30 administering to the mammal a number of second individual doses of the antagonist, wherein the second individual doses are administered less frequently than the first individual doses.

Trial Design

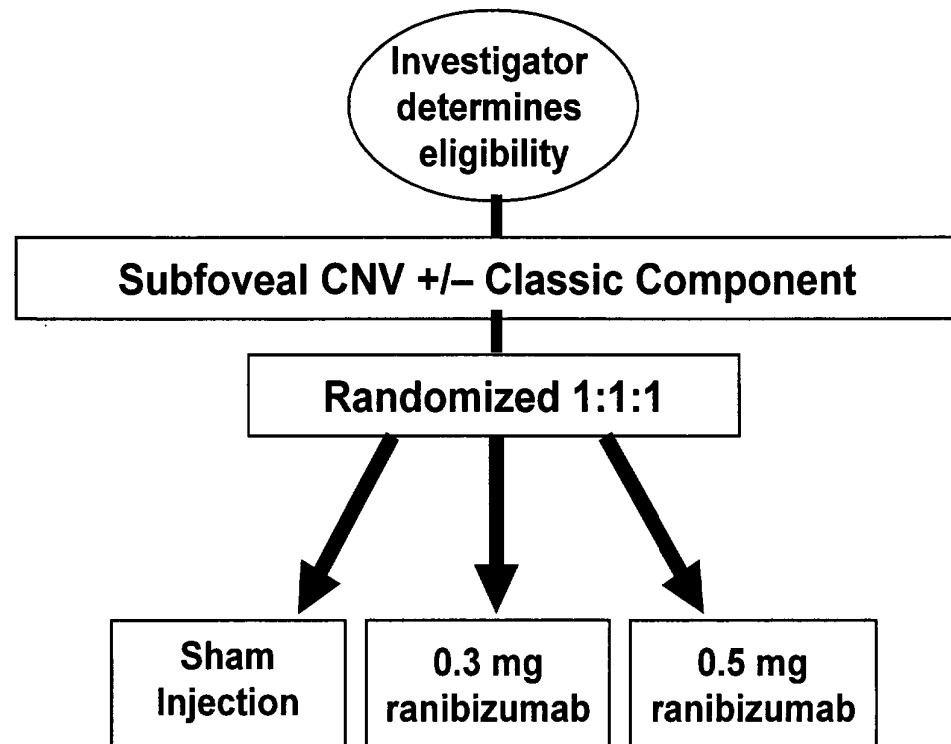


Figure 1

Treatment Schema

 = 0.3mg ranibizumab
  = 0.5mg ranibizumab
  = Sham Injection

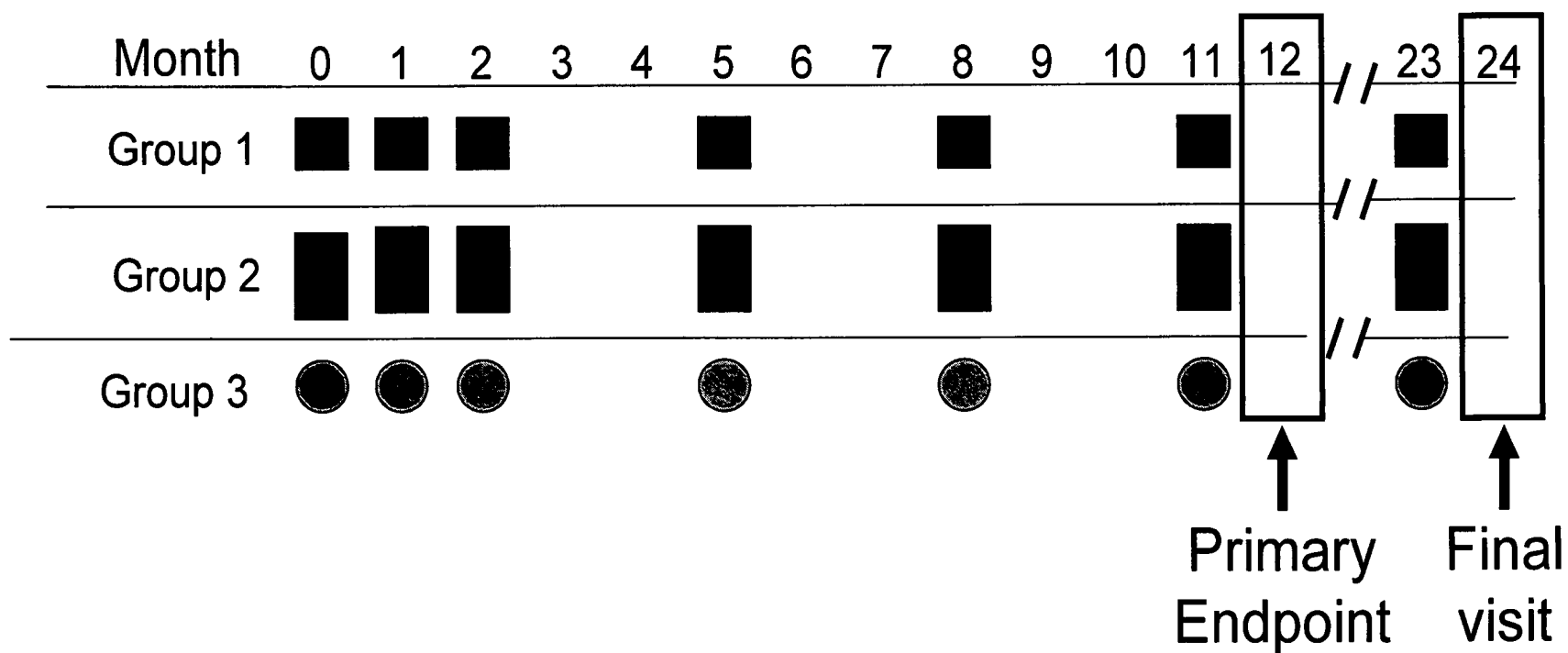


Figure 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/038006

A. CLASSIFICATION OF SUBJECT MATTER

A61K39/00

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KRZYSTOLIK MAGDALENA G ET AL: "Prevention of experimental choroidal neovascularization with intravitreal anti-vascular endothelial growth factor antibody fragment" ARCHIVES OF OPHTHALMOLOGY, vol. 120, no. 3, March 2002 (2002-03), pages 338-346, XP009061383 ISSN: 0003-9950 page 339; figures 4,5; tables 1,4 ----- -/--	1-21

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

10 February 2006

Date of mailing of the international search report

17/03/2006

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Trommsdorff, M

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2005/038006

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EARL R. NICOLS: "AAO:Ranibizumab(rhuFab) May Improve Vision in Age-Related Macular Degeneration" INTERNET ARTICLE, 'Online! 24 November 2003 (2003-11-24), XP002366839 Retrieved from the Internet: URL:http://www.pslgroup.com/dg/23f2aa.htm> 'retrieved on 2006-02-08! the whole document -----	1-21
Y	WO 98/45331 A (GENENTECH, INC; BACA, MANUEL; WELLS, JAMES, A; PRESTA, LEONARD, G; LOW) 15 October 1998 (1998-10-15) page 45 - page 46; table 14 -----	1-21
Y	WO 96/30046 A (GENENTECH, INC) 3 October 1996 (1996-10-03) page 14 - page 15; claims 1-5 -----	1-21
Y	THE EYETECH STUDY GROUP ET AL: "Anti-vascular endothelial growth factor therapy for subfoveal choroidal neovascularization secondary to age-related macular degeneration: Phase II study results." OPHTHALMOLOGY, vol. 110, no. 5, May 2003 (2003-05), pages 979-986, XP002366840 ISSN: 0161-6420 table 1A tables 1A,2,3 -----	1,2,4, 11-15, 18,21
P,Y	ANONYMOUS: "EINE MULTIZENTRISCHE,RANDOMISIERTE,DOPPELT MASKIERTE,WIRKSTOFF-KONTROLIERTE PHASE III STUDIE ZUR WIRKSAMHEIT UND SICHERHEIT VON RHUFAB V2 (RHANIBIZUMAB) IM VERGLEICH MIT EINER PHOTODYNAMISCHEN VERTEPORFIN-THERAPIE (VISUDYNE) AN PATIENTEN MIT VORWIEGEND KLASSISCHER SUBFOVEALER NEOVASKULÄRER ..." INTERNET ARTICLE - ANCHOR-STUDIE, 'Online! XP002366841 Retrieved from the Internet: URL:http://www.medizin.uni-koeln.de/kliniken/augenklinik/angiolab/klinischestudien/anchor.shtml?print> 'retrieved on 2006-02-09! the whole document ----- -/--	1-21

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2005/038006

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CAMPOCHIARO PETER ANTHONY ET AL: "Ocular neovascularization: A valuable model system." ONCOGENE, vol. 22, no. 42, 29 September 2003 (2003-09-29), pages 6537-6548, XP002366842 ISSN: 0950-9232 the whole document -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/038006

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: —
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 1-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.2

The present claims 1-5, 11-21 encompass compounds defined only by their desired function, contrary to the requirements of clarity of Article 6 PCT, because the result-to-be-achieved type of definition does not allow the scope of the claim to be ascertained. The fact that any compound could be screened does not overcome this objection, as the skilled person would not have knowledge beforehand as to whether it would fall within the scope claimed, except for the compounds disclosed in the description which are also structurally defined, see p.6-7. Undue experimentation would be required to screen compounds randomly. This non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search for claims 1-5, 11-21. The search of said claims was consequently restricted to antibodies against VEGF or structurally well defined other antagonists (such as e.g., aptamers disclosed in the art).

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2005/038006

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9845331	A	15-10-1998	AT 293640 T 15-05-2005
			AU 743758 B2 07-02-2002
			AU 7100798 A 30-10-1998
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			DE 69829891 T2 06-10-2005
			DE 122005000026 I1 04-08-2005
			DE 122005000050 I1 29-12-2005
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			TR 9903123 T2 22-05-2000
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			DE 69634079 D1 27-01-2005
			DE 69634079 T2 19-01-2006
			EP 0817648 A1 14-01-1998
			ES 2233967 T3 16-06-2005
			IL 117645 A 31-08-2005
			JP 11502853 T 09-03-1999
			NZ 305699 A 28-02-2000
			PT 817648 T 29-04-2005

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
6 January 2005 (06.01.2005)

PCT

(10) International Publication Number
WO 2005/000895 A2

- (51) International Patent Classification⁷: **C07K 14/71**
- (21) International Application Number:
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- (26) Publication Language: English
- (30) Priority Data:
10/609,775 30 June 2003 (30.06.2003) US
- (71) Applicant (for all designated States except US): **REGENERON PHARMACEUTICALS, INC.** [US/US]; 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US).
- (72) Inventors; and
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- (74) Agent: **VALETA, Gregg**; Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US).
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- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: VEGF TRAPS AND THERAPEUTIC USES THEREOF

(57) Abstract: Nucleic acid molecules and multimeric proteins capable of binding vascular endothelial growth factor (VEGF). VEGF traps are disclosed which are therapeutically useful for treating VEGF-associated conditions and diseases, and are specifically designed for local administration to specific organs, tissues, and/or cells.

VEGF TRAPS AND THERAPEUTIC USES THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The invention encompasses fusion polypeptides capable of binding vascular endothelial cell growth factor (VEGF), VEGF family members, and splice variants with specifically desirable characteristics, as well as therapeutic methods of use.

BRIEF SUMMARY OF THE INVENTION

[0002] In a first aspect, the invention features an isolated nucleic acid molecule encoding a fusion polypeptide comprising receptor components $(R1R2)_X$ and/or $(R1R3)_Y$, wherein R1 is vascular endothelial cell growth factor (VEGF) receptor component Ig domain 2 of Flt-1 (Flt1D2), R2 is VEGF receptor component Ig domain 3 of Flk-1 (Flk1D3), and R3 is VEGF receptor component Ig domain 3 of Flt-4 (Flt1D3 or R3), and wherein $X \geq 1$ and $Y \geq 1$.

[0003] In a related second aspect, the invention features a monomeric VEGF trap or fusion polypeptide comprising VEGF receptor components $(R1R2)_X$ and/or $(R1R3)_Y$ wherein $X \geq 1$, $Y \geq 1$, and R1, R2, and R3 are as defined above. The VEGF receptor components R1, R2, and R3, may be connected directly to each other or connected via one or more spacer sequences. In one specific embodiment, the monomeric VEGF trap is $(R1R2)_X$, where $X=2$. In a more specific embodiment, the monomeric VEGF trap is SEQ ID NO:24, or a functionally equivalent amino acid variant thereof. The invention encompasses a monomeric VEGF trap consisting essentially of VEGF receptor components $(R1R2)_X$ and/or $(R1R3)_Y$, and functionally equivalent amino acid variants thereof.

[0004] In a third aspect, the invention features an isolated nucleic acid molecule encoding a fusion polypeptide comprising VEGF receptor components $(R1R2)_X$ and/or $(R1R3)_Y$, and a fusion partner (FP) component selected from the group consisting of a multimerizing component (MC), a serum protein, or a molecule capable of binding a serum protein. In a preferred embodiment, FP is a multimerizing component (MC) capable of interacting with a multimerizing component on another fusion polypeptide to form a multimeric structure, e.g., a dimer or trimer. Most preferably, the MC is selected from the group consisting of (i) a multimerizing component comprising a cleavable region (C-region), (ii) a truncated multimerizing component, (iii) an amino acid sequence between 1 to about 200 amino acids in length having at least one cysteine residue, (iv) a leucine zipper, (v) a helix loop motif, (vi) a coil-coil motif, and (vii) an immunoglobulin domain. Further encompassed are fusion polypeptides consisting essentially of $(R1R2)_X$ and/or $(R1R3)_Y$, and FP. In a preferred embodiment, the fusion polypeptide consists essentially of $(R1R2)_X$ and MC.

[0005] In a fourth aspect, the invention features a fusion polypeptide comprising VEGF receptor components $(R1R2)_X$ and/or $(R1R3)_Y$, and FP, as described above. The receptor components may be arranged in different orders, for example, $(R1R2)_X$ -FP; $(R1R2)_X$ -FP- $(R1R2)_X$; FP- $(R2R1)_X$, etc. The components of the fusion polypeptide may be connected directly to each other, or connected via a spacer sequence.

[0006] In a fifth aspect, the invention features a VEGF trap, comprising a multimer of two or more fusion polypeptides consisting of VEGF receptor components (R1R2)_x and/or (R1R3)_y, and FP, wherein the FP component is a multimerizing component (MC) comprising a C-region. The C-region may be naturally occurring or artificial, and may occur at any point within the multimerizing component, and functions to allow cleavage of a parent MC to a truncated MC. A VEGF trap composed of two or more fusion polypeptides having at least one truncated MC is termed a "truncated mini-trap."

[0007] The C-region may be created in MC by insertion, deletion, or mutation, such that an enzymatically or chemically cleavable site is created. The C-region may be created in any MC and at any position within the MC; preferably, the C-region is created in a full length Fc domain, or a fragment thereof, or a C_H3 domain. The C-region may be a site cleavable by an enzyme, such as, thrombin, ficin, pepsin, matrilysin, or prolidase or cleavable chemically by, for example, formic acid or CuCl₂.

[0008] In a sixth related aspect, the invention features a truncated VEGF mini-trap which is a multimeric protein comprising two or more fusion polypeptides consisting of (R1R2)_x and/or (R1R3)_y and a multimerizing component which is a truncated by cleavage from a parent MC comprising a C-region (tMC).

[0009] In a seventh aspect, the invention features a fusion polypeptide consisting of VEGF receptor components (R1R2)_x and/or (R1R3)_y and a MC, wherein the MC is an amino acid sequence between 1 to about 200 amino acids in length comprising at least one cysteine residue, wherein the at least one cysteine residue is capable of forming a disulfide bond with a cysteine residue present in the MC of another fusion polypeptide (cMC). In a preferred embodiment, cMC is an amino acid sequence between 1-50 amino acids in length comprising at least one cysteine residue. In a more preferred embodiment, cMC is an amino acid sequence between 1-15 amino acids in length comprising at least one amino acid. In an even more preferred embodiment, cMC is an amino acid sequence between 1-10 amino acids in length comprising 1-2 cysteine residues. One exemplification of this embodiment of the invention is shown in SEQ ID NO:27 having a signal sequence (1-26) followed by R1 (27-129) and R2 (130-231) components, followed by a nine amino acid sequence ending in a cysteine residue. In another embodiment, shown in SEQ ID NO:28, a signal sequence (1-26) is followed by R1 (27-129) and R2 (130-231) components, followed by a six amino acid sequence ending in a cysteine residue.

[0010] In an eighth aspect, the invention features a VEGF mini-trap, comprising a multimer of two or more fusion polypeptides consisting of (R1R2)_x and/or (R1R3)_y and a cMC. In a more specific embodiment, the mini-trap is a dimer. One exemplification of this embodiment of the mini-trap of the invention is a dimer of the fusion polypeptide shown in SEQ ID NO:2, wherein each fusion polypeptide (R1R2-cMC) has a molecular weight of 23.0 kD and a pI of 9.22.

[0011] In another embodiment, cMC is 4 amino acids in length consisting of two cysteine residues, for example, XCXC (SEQ ID NO:3). In one exemplification of this embodiment of the invention, the mini-trap consists of the VEGF receptor components of the invention, and a cMC consisting of ACGC (SEQ ID NO:4). One exemplification of this embodiment of the mini-trap of the invention is

a dimer of the fusion polypeptide shown in SEQ ID NO:5, wherein each monomer has a molecular weight of 23.2 kD and a pI of 9.22. Another exemplification of this embodiment of the invention is shown in SEQ ID NO:26 having a signal sequence (1-26) followed by R1 (27-129) and R2 (130-231) components, followed by a nine amino acid sequence ending in CPPC.

[0012] In all embodiments of the VEGF trap of the invention (including truncated VEGF mini-trap, VEGF mini-traps, and monomeric VEGF mini-traps), a signal sequence (S) may be included at the beginning (or N-terminus) of the fusion polypeptide of the invention. The signal sequence may be native to the cell, recombinant, or synthetic. When a signal sequence is attached to the N-terminus of a first receptor component, thus a fusion polypeptide may be designated as, for example, S-(R1R2)_x.

[0013] The components of the fusion polypeptide may be connected directly to each other or be connected via spacers. In specific embodiments, one or more receptor and/or fusion partner components of the fusion polypeptide are connected directly to each other without spacers. In other embodiments, one or more receptor and/or fusion partner components are connected with spacers.

[0014] The invention encompasses vectors comprising the nucleic acid molecules of the invention, including expression vectors comprising the nucleic acid molecule operatively linked to an expression control sequence. The invention further encompasses host-vector systems for the production of a fusion polypeptide which comprise the expression vector, in a suitable host cell; host-vector systems wherein the suitable host cell is a bacterial, yeast, insect, mammalian cell; an *E. coli* cell, or a COS or CHO cell. Additional encompassed are VEGF traps of the invention modified by acetylation or pegylation. Methods for acetylating or pegylating a protein are well known in the art.

[0015] In a related ninth aspect, the invention features a method of producing a VEGF trap of the invention, comprising culturing a host cell transfected with a vector comprising a nucleic acid sequence of the invention, under conditions suitable for expression of the protein from the host cell, and recovering the fusion polypeptides so produced.

[0016] The VEGF traps of the invention are therapeutically useful for treating any disease or condition which is improved, ameliorated, or inhibited by removal, inhibition, or reduction of VEGF. A non-exhaustive list of specific conditions improved by inhibition or reduction of VEGF include, for example, undesirable plasma leakage or vascular permeability, undesirable blood vessel growth, e.g., such as in a tumor, edema associated with inflammatory disorders such as psoriasis or arthritis, including rheumatoid arthritis; asthma; generalized edema associated with burns; ascites and pleural effusion associated with tumors, inflammation or trauma; chronic airway inflammation; asthma; capillary leak syndrome; sepsis; kidney disease associated with increased leakage of protein; pancreatic ductal adenocarcinoma (PDAC) and eye disorders such as age related macular degeneration and diabetic retinopathy. The VEGF mini-trap is particularly useful in treatment of eye disorders, and as an adjuvant to eye surgeries, including glaucoma surgery; and the treatment of intra-ocular tumors, such as for example, uveal melanoma, retinoblastoma, via intravitreal delivery.

[0017] Accordingly, in a tenth aspect, the invention features a therapeutic method for the treatment of a VEGF-related disease or condition, comprising administering a VEGF trap of the invention to a subject suffering from a VEGF-related disease or condition. Although any mammal

can be treated by the therapeutic methods of the invention, the subject is preferably a human patient suffering from or at risk of suffering from a condition or disease which can be improved, ameliorated, inhibited or treated with a VEGF trap.

[0018] In a eleventh aspect, the invention further features diagnostic and prognostic methods, as well as kits for detecting, quantitating, and/or monitoring VEGF with the mini-traps of the invention.

[0019] In a twelfth aspect, the invention features pharmaceutical compositions comprising a VEGF trap of the invention with a pharmaceutically acceptable carrier. Such pharmaceutical compositions may comprise a dimeric fusion polypeptide trap, or nucleic acids encoding the fusion polypeptide. The mini-traps of the invention find specific uses in conditions in which a VEGF trap with reduced serum half life (e.g., faster clearance), and/or increased tissue penetration due to smaller size is desirable. Specific applications for the VEGF mini-trap include, for example, diseases where local administration to a specific tissue or cell is desirable. Examples of such a condition or disease are ocular diseases of the eye.

[0020] Other objects and advantages will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION OF THE INVENTION

[0021] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only the appended claims.

[0022] As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0023] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to describe the methods and/or materials in connection with which the publications are cited.

General Description

[0024] The invention encompasses a VEGF trap capable of binding and inhibiting VEGF activity which is a monomer or multimer of one or more fusion polypeptides. The molecules of the invention bind and inhibit the biological action of VEGF and/or the physiological reaction or response. For a description of VEGF-receptor-based antagonist VEGF traps Flt1D2.Flk1D3.Fc Δ C1(a) (SEQ ID NOs:7-8) and VEGFR1R2-Fc Δ C1(a) (SEQ ID NOs:9-10), see PCT WO/0075319, the contents of which is incorporated in its entirety herein by reference.

[0025] The mini-trap of the invention is smaller than the full sized trap, e.g., about 50 - 60 kD

versus 120 kD of the parent trap, and include monomeric traps consisting essentially of VEGF receptor domains (R1R2)_x, (R1R3)_y, or combinations thereof, traps generated by cleavage of a portion of a parent multimerized trap having a fusion partner component which is a multimerizing component (MC) containing a cleavage region (C-region); or by attaching a cysteine residue or amino acid sequence containing one or more cysteine residues to or between receptor component domains. In specific embodiments, the mini-trap of the invention is less than about 60 kD as measured by SDS-PAGE analysis; more preferably, about 50 kD; even more preferably about 20-30 kD; or is about 25 kD and capable of binding VEGF with an affinity comparable to a full-sized parent trap described in PCT/US00/14142.

Nucleic Acid Constructs and Expression

[0026] The present invention provides for the construction of nucleic acid molecules encoding fusion polypeptides capable of binding VEGF alone or multimerized VEGF traps. The nucleic acid molecules of the invention may encode wild-type R1, R2, and/or R3 receptor components, or functionally equivalent variants thereof. Amino acid sequence variants of the R1, R2 and/or R3 receptor components of the traps of the invention may also be prepared by creating mutations in the encoding nucleic acid molecules. Such variants include, for example, deletions from, or insertions or substitutions of, amino acid residues within the amino acid sequence of R1, R2 and/or R3. Any combination of deletion, insertion, and substitution may be made to arrive at a final construct, provided that the final construct possesses the ability to bind and inhibit VEGF.

[0027] These nucleic acid molecules are inserted into a vector that is able to express the fusion polypeptides when introduced into an appropriate host cell. Appropriate host cells include, but are not limited to, bacterial, yeast, insect, and mammalian cells. Any of the methods known to one skilled in the art for the insertion of DNA fragments into a vector may be used to construct expression vectors encoding the fusion polypeptides of the invention under control of transcriptional/translational control signals.

[0028] Expression of the nucleic acid molecules of the invention may be regulated by a second nucleic acid sequence so that the molecule is expressed in a host transformed with the recombinant DNA molecule. For example, expression may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression of the chimeric polypeptide molecules include, but are not limited to, a long terminal repeat (Squinto et al. (1991) Cell 65:1-20); SV40 early promoter region, CMV, M-MuLV, thymidine kinase promoter, the regulatory sequences of the metallothioneine gene; prokaryotic expression vectors such as the b-lactamase promoter, or the tac promoter (see also Scientific American (1980) 242:74-94); promoter elements from yeast or other fungi such as Gal 4 promoter, ADH, PGK, alkaline phosphatase, and tissue-specific transcriptional control regions derived from genes such as elastase I.

[0029] Expression vectors capable of being replicated in a bacterial or eukaryotic host comprising the nucleic acid molecules of the invention are used to transfect the host and thereby direct expression of such nucleic acids to produce the fusion polypeptides of the invention, which form traps capable of binding to VEGF. Transfected cells may transiently or, preferably, constitutively

and permanently express the VEGF traps of the invention.

[0030] The traps of the invention may be purified by any technique which allows for the subsequent formation of a stable, biologically active trap. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis (see, for example, US Patent No. 5,663,304). In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

VEGF Receptor Components

[0031] The VEGF receptor components of the VEGF mini trap consist of the Ig domain 2 of Flt-1 (Flt1D2) (R1), the Ig domain 3 of Flk-1 (Flk1D3) (R2) (together, R1R2), and/or R1 and Ig domain 3 of Flt-4 (Flt1D3) (R3) (together, R1R3). The term "Ig domain" of Flt-1, Flt-4, or Flk-1 is intended to encompass not only the complete wild-type domain, but also insertional, deletional, and/or substitutional variants thereof which substantially retain the functional characteristics of the intact domain. It will be readily apparent to one of skill in the art that numerous variants of the above Ig domains can be obtained which will retain substantially the same functional characteristics as the wild-type domain.

[0032] The term "functional equivalents" when used in reference to R1, R2, or R3, is intended to encompass an R1, R2, or R3 domain with at least one alteration, e.g., a deletion, addition, and/or substitution, which retains substantially the same functional characteristics as does the wild type R1, R2, or R3 domain, that is, a substantially equivalent binding to VEGF. It will be appreciated that various amino acid substitutions can be made in R1, R2, or R3 without departing from the spirit of the invention with respect to the ability of these receptor components to bind and inactivate VEGF. The functional characteristics of the traps of the invention may be determined by any suitable screening assay known to the art for measuring the desired characteristic. Examples of such assays are described in the experimental section below which allow determination of binding characteristics of the traps for VEGF (K_d), as well as their half-life of dissociation of the trap-ligand complex ($T_{1/2}$). Other assays, for example, a change in the ability to specifically bind to VEGF can be measured by a competition-type VEGF binding assay. Modifications of protein properties such as thermal stability, hydrophobicity, susceptibility to proteolytic degradation, or tendency to aggregate may be measured by methods known to those of skill in the art.

[0033] The components of the fusion polypeptide may be connected directly to each other or be connected via spacers. Generally, the term "spacer" (or linker) means one or more molecules, e.g., nucleic acids or amino acids, or non-peptide moieties, such as polyethylene glycol, which may be inserted between one or more component domains. For example, spacer sequences may be used to provide a desirable site of interest between components for ease of manipulation. A spacer may also be provided to enhance expression of the fusion polypeptide from a host cell, to decrease steric hindrance such that the component may assume its optimal tertiary structure and/or interact appropriately with its target molecule. For spacers and methods of identifying desirable spacers, see,

for example, George et al. (2003) Protein Engineering 15:871-879, herein specifically incorporated by reference. A spacer sequence may include one or more amino acids naturally connected to a receptor component, or may be an added sequence used to enhance expression of the fusion polypeptides, provide specifically desired sites of interest, allow component domains to form optimal tertiary structures and/or to enhance the interaction of a component with its target molecule. In one embodiment, the spacer comprises one or more peptide sequences between one or more components which is (are) between 1-100 amino acids, preferably 1-25.

[0034] In the most specific embodiments, R1 is amino acids 27-126 of SEQ ID NO:8, or 1-126 of SEQ ID NO:8 (including the signal sequence 1-26); or amino acids 27-129 of SEQ ID NO:10, or 1-129 of SEQ ID NO:10 (including the signal sequence at 1-26). In the most specific embodiments, R2 is amino acids 127-228 of SEQ ID NO:8, or amino acids 130-231 of SEQ ID NO:10. In the most specific embodiments, R3 is amino acids 127-225 of SEQ ID NO: 13 (without a signal sequence). When, for example, R2 is placed at the N-terminus of the fusion polypeptide, a signal sequence may desirably precede the receptor component. The receptor component(s) attached to the multimerizing component may further comprise a spacer component, for example, the GPG sequence of amino acids 229-231 of SEQ ID NO:7.

Fusion Partner and Multimerizing Components

[0035] The fusion partner is any component that enhances the functionality of the fusion polypeptide. Thus, for example, an fusion partner may enhance the biological activity of the fusion polypeptide, aid in its production and/or recovery, or enhance a pharmacological property or the pharmacokinetic profile of the fusion polypeptide by, for example, enhancing its serum half-life, tissue penetrability, lack of immunogenicity, or stability. In preferred embodiments, the fusion partner is selected from the group consisting of a multimerizing component, a serum protein, or a molecule capable of binding a serum protein.

[0036] When the fusion partner is a serum protein or fragment thereof, it is selected from the group consisting of α -1-microglobulin, AGP-1, orosomucoid, α -1-acid glycoprotein, vitamin D binding protein (DBP), hemopexin, human serum albumin (hSA), transferrin, ferritin, afamin, haptoglobin, α -fetoprotein thyroglobulin, α -2-HS-glycoprotein, β -2-glycoprotein, hyaluronan-binding protein, syntaxin, C1R, C1q a chain, galectin3-Mac2 binding protein, fibrinogen, polymeric Ig receptor (PIGR), α -2-macroglobulin, urea transport protein, haptoglobin, IGFBPs, macrophage scavenger receptors, fibronectin, giantin, Fc, α -1-antichymotrypsin, α -1-antitrypsin, antithrombin III, apolipoprotein A-I, apolipoprotein B, β -2-microglobulin, ceruloplasmin, complement component C3 or C4, CI esterase inhibitor, C-reactive protein, cystatin C, and protein C. In a more specific embodiment, fusion partner is selected from the group consisting of α -1-microglobulin, AGP-1, orosomucoid, α -1-acid glycoprotein, vitamin D binding protein (DBP), hemopexin, human serum albumin (hSA), afamin, and haptoglobin. The inclusion of a fusion partner component may extend the serum half-life of the fusion polypeptide of the invention when desired. See, for example, US Patent Nos. 6,423,512, 5,876,969, 6,593,295, and 6,548,653, herein specifically incorporated by

reference in their entirety, for examples of serum albumin fusion polypeptides. hSA is widely distributed throughout the body, particularly in the intestinal and blood components, and has an important role in the maintenance of osmolarity and plasma volume. It is slowly cleared in the liver, and typically has an *in vivo* half-life of 14-20 days in humans (Waldmann et al. (1977) Albumin, Structure Function and Uses; Pergamon Press; pp. 255-275).

[0037] When a fusion partner is a molecule capable of binding a serum protein, the molecule may be a synthetic small molecule, a lipid or liposome, a nucleic acid, including a synthetic nucleic acid such as an aptomer, a peptide, or an oligosaccharide. The molecule may further be a protein, such as, for example, FcγR1, FcγR2, FcγR3, polymeric Ig receptor (PIGR), ScFv, and other antibody fragments specific for a serum protein.

[0038] When the fusion partner is a multimerizing component (MC), it is any natural or synthetic sequence capable of interacting with another MC to form a higher order structure, e.g., a dimer, a trimer, etc. Suitable MCs may include a leucine zipper, including leucine zipper domains derived from c-jun or c-fos; sequences derived from the constant regions of kappa or lambda light chains; synthetic sequences such as helix-loop-helix motifs (Müller et al. (1998) FEBS Lett. 432:45-49), coil-coil motifs, etc., or other generally accepted multimerizing domains known to the art. In some embodiments, the fusion component comprises an immunoglobulin-derived domain from, for example, human IgG, IgM or IgA. In specific embodiments, the immunoglobulin-derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. The Fc domain of IgG may be selected from the isotypes IgG1, IgG2, IgG3, and IgG4, as well as any allotype within each isotype group. In one example of the VEGF trap of the invention, the multimerizing component is an IgG4 Fc domain (SEQ ID NO:29).

Generation of Truncated VEGF Mini-Traps

[0039] In one embodiment of the trap of the invention, a truncated VEGF mini-trap comprising two or more fusion polypeptides of the invention, is generated by subjecting a parent trap having C-region-containing MCs to conditions under which one or more of the C-region-containing MCs is (are) cleaved. The resulting truncated mini-trap may be a full and partial cleavage product of a parent trap.

[0040] The C-region-containing MC may be any MC capable of interacting with another MC to form a higher order structure, e.g., a dimer or a trimer. The C-region may be created within an MC at any desired location. In light of the guidance provided in the examples below, one of skill in the art would be able to select a desired site for creation of a C-region based on the desired properties of the resulting truncated traps, e.g., molecular weight, monomeric or dimeric, etc.

[0041] In a specific embodiment, the C-region is a thrombin cleavage site (LVPRGS) (SEQID NO:6) inserted into an FcΔC1 domain following the N-terminal CPPC sequence (SEQ ID NO:1). In this embodiment, a full-sized parent VEGF trap construct is expressed in a cell as an Fc-tagged protein, thus allowing capture and purification by, for example, a Protein A column. Following formation of a dimer and covalent bonding between one or both of the cysteine residues of the CPPC sequence

(SEQ ID NO:1), the dimer is exposed to thrombin under conditions which cleave one or both of the Fc Δ C1 domains such that truncated dimeric mini-traps are generated, having a molecular weight of approximately 50 kD – 90 kD, and has an affinity for VEGF comparable to that of the parent trap. The conditions of cleavage may be controlled by one of skill in the art to favor formation of the partial cleavage product or the fully cleaved product, the choice of cleavage conditions selected by desire for a particular product having specific properties such as molecular weight.

[0042] In a specific embodiment, the C-region is a thrombin cleavage site (LVPRGS) (SEQ ID NO:6) inserted into an Fc Δ C1 domain N-terminal to the CPPC sequence (SEQ ID NO:1). Following formation of a dimer and covalent bonding between one or both of the cysteine residues of the CPPC sequence (SEQ ID NO:1), the dimer is exposed to thrombin under conditions in which one or both of the Fc Δ C1 domain occur and truncated monomeric mini-traps are generated. The monomeric truncated mini-trap thus generated comprises a receptor component, and a small fragment of the Fc, and is approximately 25 kD in size and exhibits a reduced affinity for VEGF relative to the truncated dimeric trap and the full length parent trap. A similar monomeric trap produced as a recombinant protein has been shown to have a K_D of about 1 nM.

Generation of VEGF Mini-Traps

[0043] In one embodiment, the invention features VEGF mini-traps having one or more receptor component domains (R1R2)_X and/or R1R3)_Y, wherein $X \geq 1$, $Y \geq 1$, and R1, R2, and R3 are as defined above, and optionally, a fusion partner which is preferably a MC domain which is an amino acid sequence between 1 to about 200 amino acids in length comprising at least one cysteine residue, wherein the at least one cysteine residue is capable of forming a disulfide bond with a cysteine residue present in the MC of another fusion polypeptide (cMC). The cMC may occur at the N-terminus or C-terminus of a fusion polypeptide, or between two receptor component domains. In one specific embodiment, cysteine is added to the C-terminus of a VEGF receptor component, e.g., R1R2_C, which allows the fusion polypeptide to form covalent dimers through formation of a covalent disulfide bond between the cysteine residue at the C-terminus of one fusion polypeptide and the cysteine residue at the C-terminus of another fusion polypeptide. In this exemplification, the mini-trap is a dimer of the fusion polypeptide shown in SEQ ID NO:2, wherein each fusion polypeptide (R1R2-cMC or R1R2_C) has a molecular weight of about 23.0 kD.

[0044] In another embodiment, the cMC is a sequence of 4 amino acids (XXXX) (SEQ ID NO:11) wherein X is any amino acid and the sequence comprises at least one cysteine residue. In a specific embodiment, the cMC is added to the C-terminus of a receptor component domain. In a more specific embodiment, the 4 amino acid sequence is ACGC (SEQ ID NO:4) and the cMC forms two disulfide bonds with the cysteine residues present in a second fusion polypeptide. As shown below (Table 2), both the exemplified mini-traps exhibit an affinity for VEGF comparable to the parent trap.

Therapeutic Uses

[0045] The VEGF mini-traps of the invention are therapeutically useful for treating any disease or

condition which is improved, ameliorated, inhibited or prevented by removal, inhibition, or reduction of VEGF. A non-exhaustive list of specific conditions improved by inhibition or reduction of VEGF include, clinical conditions that are characterized by excessive vascular endothelial cell proliferation, vascular permeability, edema or inflammation such as brain edema associated with injury, stroke or tumor; edema associated with inflammatory disorders such as psoriasis or arthritis, including rheumatoid arthritis; asthma; generalized edema associated with burns; ascites and pleural effusion associated with tumors, inflammation or trauma; chronic airway inflammation; capillary leak syndrome; sepsis; kidney disease associated with increased leakage of protein; and eye disorders such as age related macular degeneration and diabetic retinopathy.

[0046] The compositions of the invention are therapeutically useful for treating a wide variety of diseases associated with increased VEGF levels. For example, exaggerated Th2 inflammation and airway remodeling are characteristic in the pathogenesis of asthma (see, for example, Elias et al. (1999) *J. Clin. Invest.* 104:1001-6). Elevated VEGF levels have been detected in tissues and biologic samples from patients with asthma, which correlate directly with disease activity (Lee et al. (2001) *J. Allergy Clin. Immunol.* 107:1106-1108) and inversely with airway caliber and airway responsiveness. Further, VEGF has been postulated to contribute to asthmatic tissue edema.

[0047] Another disease associated with increased VEGF is pancreatic ductal adenocarcinoma (PDAC). This malignancy often exhibits enhanced foci of endothelial cell proliferation and frequently overexpresses VEGF (Ferrara (1999) *J. Mol. Med.* 77:527-543). PDAC is responsible for over 20% of deaths due to gastrointestinal malignancies, making it the fourth most common cause of cancer-related mortality in the U.S. and other industrialized countries. Experimental evidence supports an important role for VEGF in pancreatic cancer, thus a VEGF inhibitor has promise as a therapeutic to attenuate intrapancreatic tumor growth and regional and distal metastasis.

[0048] A smaller, non-glycosylated mini-trap expressed in *E. coli* (Example 4), a glycosylated mini-trap expressed in CHO cells (Example 5), or a receptor-based monomeric trap (Example 6) has optimized characteristics for local/intra-vitreous delivery, ie. a shorter serum half life for faster clearance and minimizing unwanted systemic exposure. In addition due to its smaller size, the mini-trap has the ability to penetrate through the inner-limiting membrane (ILM) in the eye, and diffuse through the vitreous to the retina/retinal pigment epithelial (RPE) layer which will help to treat retinal disease. Additionally, the mini-trap can be used for local administration for the treatment of ocular disease such as choroidal neovascularization, diabetic macular edema, proliferative diabetic retinopathy, corneal neovascularization/transplant rejection. Still further, the mini-trap can be used in any situation where transient (short-term) blocking of VEGF is required, e.g., to avoid chronic exposure to VEGF blockade, such as, for example, in the treatment of psoriasis.

[0049] A serious problem leading to failure following glaucoma surgery is early inflammation and angiogenesis, as well as too aggressive wound healing. Accordingly, the VEGF traps of the invention may be usefully employed as an adjuvant to glaucoma surgery to prevent early hem- and lymphangiogenesis and macrophage recruitment to the filterig bleb after glaucoma surgery, and improve surgical outcome.

Combination Therapies

[0050] In numerous embodiments, a VEGF trap may be administered in combination with one or more additional compounds or therapies, including a second VEGF trap molecule, a chemotherapeutic agent, surgery, catheter devices, and radiation. Combination therapy includes administration of a single pharmaceutical dosage formulation which contains a VEGF trap and one or more additional agents; as well as administration of a VEGF trap and one or more additional agent(s) in its own separate pharmaceutical dosage formulation. For example, a VEGF trap and a cytotoxic agent, a chemotherapeutic agent or a growth inhibitory agent can be administered to the patient together in a single dosage composition such as a combined formulation, or each agent can be administered in a separate dosage formulation. Where separate dosage formulations are used, the VEGF-specific fusion polypeptide of the invention and one or more additional agents can be administered concurrently, or at separately staggered times, i.e., sequentially.

[0051] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. I^{131} , I^{125} , Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

[0052] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (Cytosan®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitioestanol, mepitioestane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®;

razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel (Taxol®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (Taxotere®; Aventis Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY 117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0053] A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially a cancer cell either *in vitro* or *in vivo*. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), Taxol®, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C.

Methods of Administration

[0054] The invention provides methods of treatment comprising administering to a subject an effective amount of a VEGF trap of the invention. In a preferred aspect, the trap is substantially purified (*e.g.*, substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably a mammal, and most preferably a human.

[0055] Various delivery systems are known and can be used to administer an agent of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction can be enteral or parenteral and include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, intraocular, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. Administration can be acute or chronic (*e.g.* daily, weekly, monthly, etc.) or in combination with other agents. Pulmonary administration can also be employed,

e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0056] In another embodiment, the active agent can be delivered in a vesicle, in particular a liposome, in a controlled release system, or in a pump. In another embodiment where the active agent of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see, for example, U.S. Patent No. 4,980,286), by direct injection, or by use of microparticle bombardment, or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliet et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0057] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, *e.g.*, by injection, by means of a catheter, or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, fibers, or commercial skin substitutes.

[0058] A composition useful in practicing the methods of the invention may be a liquid comprising an agent of the invention in solution, in suspension, or both. The term "solution/suspension" refers to a liquid composition where a first portion of the active agent is present in solution and a second portion of the active agent is present in particulate form, in suspension in a liquid matrix. A liquid composition also includes a gel. The liquid composition may be aqueous or in the form of an ointment. Further, the composition can take the form of a solid article that can be inserted in the eye, such as for example between the eye and eyelid or in the conjunctival sac, where the VEGF trap is released. Release from such an article is usually to the cornea, either via the lacrimal fluid, or directly to the cornea itself, with which the solid article is generally in direct contact. Solid articles suitable for implantation in the eye are generally composed primarily of bioerodible or nonbioerodible polymers. An aqueous solution and/or suspension can be in the form of eye drops. A desired dosage of the active agent can be measured by administration of a known number of drops into the eye. For example, for a drop volume of 25 μ l, administration of 1-6 drops will deliver 25-150 μ l of the composition.

[0059] An aqueous suspension or solution/suspension useful for practicing the methods of the invention may contain one or more polymers as suspending agents. Useful polymers include water-soluble polymers such as cellulosic polymers and water-insoluble polymers such as cross-linked carboxyl-containing polymers. An aqueous suspension or solution/suspension of the present invention is preferably viscous or muco-adhesive, or even more preferably, both viscous or mucoadhesive.

[0060] In another embodiment, the composition useful in practicing the methods of the invention is an *in situ* gellable aqueous composition. Such a composition comprises a gelling agent in a concentration effective to promote gelling upon contact with the eye or with lacrimal fluid. Suitable

gelling agents include but are not limited to thermosetting polymers. The term "*in situ* gellable" as used herein includes not only liquids of low viscosity that form gels upon contact with the eye or with lacrimal fluid, but also includes more viscous liquids such as semi-fluid and thixotropic gels that exhibit substantially increased viscosity or gel stiffness upon administration to the eye.

Diagnostic and Screening Methods

[0061] The VEGF traps of the invention may be used diagnostically and/or in screening methods. For example, the trap may be used to monitor levels of VEGF during a clinical study to evaluate treatment efficacy. In another embodiment, the methods and compositions of the present invention are used to screen individuals for entry into a clinical study to identify individuals having, for example, too high or too low a level of VEGF. The traps can be used in methods known in the art relating to the localization and activity of VEGF, *e.g.*, imaging, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc.

[0062] The traps of the invention may be used in *in vivo* and *in vitro* screening assay to quantify the amount of non-bound VEGF present, *e.g.*, for example, in a screening method to identify test agents able to decrease the expression of VEGF. More generally, the traps of the invention may be used in any assay or process in which quantification and/or isolation of VEGF is desired.

Pharmaceutical Compositions

[0063] The present invention also provides pharmaceutical compositions comprising a VEGF mini-trap of the invention. Such compositions comprise a therapeutically effective amount of one or more mini-traps, and a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

[0064] The VEGF mini-trap of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0065] Further more, aqueous compositions useful for practicing the methods of the invention have ophthalmically compatible pH and osmolality. One or more ophthalmically acceptable pH adjusting agents and/or buffering agents can be included in a composition of the invention, including acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, and sodium lactate; and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases, and buffers are included in an amount required to maintain pH of the composition in an ophthalmically acceptable range. One or more ophthalmically acceptable salts can be included in the composition in an amount sufficient to bring osmolality of the composition into an ophthalmically acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions.

[0066] The amount of the trap that will be effective for its intended therapeutic use can be determined by standard clinical techniques based on the present description. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. Generally, suitable dosage ranges for intravenous administration are generally about 50-5000 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0067] For systemic administration, a therapeutically effective dose can be estimated initially from *in vitro* assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Initial dosages can also be estimated from *in vivo* data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

[0068] Dosage amount and interval may be adjusted individually to provide plasma levels of the compounds that are sufficient to maintain therapeutic effect. In cases of local administration or selective uptake, the effective local concentration of the compounds may not be related to plasma concentration. One having skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

[0069] The amount of compound administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration, and the judgment of the prescribing physician. The therapy may be repeated intermittently while symptoms are detectable or even when they are not detectable. The therapy may be provided alone or in combination with other drugs.

Cellular Transfection and Gene Therapy

[0070] The present invention encompasses the use of nucleic acids encoding the fusion polypeptides of the invention for transfection of cells *in vitro* and *in vivo*. These nucleic acids can be inserted into any of a number of well-known vectors for transfection of target cells and organisms. The nucleic acids are transfected into cells *ex vivo* and *in vivo*, through the interaction of the vector and the

target cell. The compositions are administered (e.g., by injection into a muscle) to a subject in an amount sufficient to elicit a therapeutic response. An amount adequate to accomplish this is defined as "a therapeutically effective dose or amount."

[0071] In another aspect, the invention provides a method of reducing VEGF levels in a human or other animal comprising transfecting a cell with a nucleic acid encoding a fusion polypeptide of the invention, wherein the nucleic acid comprises an inducible promoter operably linked to the nucleic acid encoding the fusion polypeptide or mini-trap. For gene therapy procedures in the treatment or prevention of human disease, see for example, Van Brunt (1998) *Biotechnology* 6:1149-1154.

Kits

[0072] The invention also provides an article of manufacturing comprising packaging material and a pharmaceutical agent contained within the packaging material, wherein the pharmaceutical agent comprises at least one VEGF trap composed of two or more fusion polypeptides of the invention, and wherein the packaging material comprises a label or package insert which indicates that the VEGF-specific fusion polypeptide can be used for treating a VEGF-mediated disease or condition.

Transgenic Animals

[0073] The invention includes transgenic non-human animals expressing a trap of the invention. A transgenic animal can be produced by introducing nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. Any of the regulatory or other sequences useful in expression vectors can form part of the transgenic sequence. A tissue-specific regulatory sequence(s) can be operably linked to the transgene to direct expression of the transgene to particular cells. A transgenic non-human animal expressing a fusion polypeptide or mini-trap of the invention is useful in a variety of applications, including as a means of producing such a fusion polypeptide.. Further, the transgene may be placed under the control of an inducible promoter such that expression of the fusion polypeptide or mini-trap may be controlled by, for example, administration of a small molecule.

Specific Embodiments

[0074] In the experiments described below, smaller VEGF traps were generated and their ability to bind VEGF was investigated. Such mini-traps are preferably uses in specific applications. For example, certain conditions or diseases may be preferably treated with local administration of a VEGF trap to a specific organ, tissue, or cell, rather than by systemic administration. In one exemplification of the mini-traps of the invention, a smaller VEGF trap was generated by directed cleavage of a dimerized VEGF trap having a cleavage region (C-region) generated in a Fc domain (Example 2). The truncated trap exhibited comparable affinity for VEGF and half-life as the full-sized parent trap. Examples 3-5 describe construction of fusion polypeptides having a VEGF receptor component and a multimerizing component consisting of one or two cysteine residues. Affinity measurements showed that the non-glycosylated fusion polypeptides expressed in *E. coli* or

the glycosylated polypeptides expressed in CHO cells had comparable binding affinity for VEGF as the full-sized parent trap. Example 6 further illustrates a monomeric VEGF trap consisting of (R1R2)₂ which is capable of binding and inhibiting VEGF. Example 7 describes the construction of a VEGF mini-trap (SEQ ID NO:26) exhibiting high affinity binding for VEGF comparable to the full length trap (SEQ ID NO:10).

[0075] Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

EXAMPLES

[0076] The following example is put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Construction of Flt1D2.Flk1D3.FcΔC1(a)

[0077] The construction of a parent VEGF trap, Flt1D2.Flk1D3.FcΔC1(a) (SEQ ID NOs:7-8), VEGFR1R2.FcΔC1(a) (SEQ ID NOs:9-10), and Flt1D2.VEGFR3D3.FcΔC1(a) (SEQ ID NOs:12-13) is described in detail in PCT publication WO/0075319, herein specifically incorporated by reference in its entirety. Also described in WO/0075319 are methods of constructing and expressing nucleic acid constructs encoding VEGF traps, methods of detecting and measuring VEGF trap binding to VEGF, methods of determining the stoichiometry of VEGF binding by BIAcore analysis, and pharmacokinetic analyses.

Example 2: Thrombin-cleaved dimeric VEGF mini-trap

[0078] The VEGFR1R2.FcΔC1(a) (SEQ ID NOs:9-10) construct was modified by insertion of a thrombin cleavage following the CPPC (SEQ ID NO:1) of the Fc domain. Purified VEGF trap (5 μg) was incubated with thrombin (Novagen) in 20 mM Tris-HCl, pH 8.4, 50 mM NaCl, 2.5 mM CaCl₂ for 16 hrs at 37° C. Controls included cleavage control protein (CCP) and parent VEGF trap protein incubated without thrombin. SDS-PAGE analysis (Tris-Glycine 4-20% gel; 5 μg protein per lane) verified correct cleavage (results not shown).

[0079] Affinity determination. The K_d of binding of each VEGF trap to hVEGF₁₆₅ was determined as described in WO/0075319, for the parent VEGF trap, uncleaved VEGF trap containing a thrombin cleavage site ("uncleaved VEGF trap"), cleaved VEGF mini-trap and recombinant monomeric R1R2-myc myc his. More specifically, the ability of the traps to block VEGF₁₆₅-dependent receptor phosphorylation was determined using primary human endothelial cells (HUVECs). VEGF₁₆₅ was incubated in the presence of varying concentrations of the test traps, and the mixture was added to

HUVECs to stimulate tyrosine phosphorylation of VEGFR2. At sub-stoichiometric concentrations of VEGF trap, unbound VEGF induced receptor phosphorylation. However, at a 1:1 molar ratio of greater of a VEGF trap to ligand, complete blocking of receptor signaling was observed, establishing that a single molecule of a trap dimer is capable of blocking a single molecule of human VEGF₁₆₅. Thus, the high binding affinity of the VEGF trap for VEGF results in formation of a complex that prevents VEGF from interaction with cell surface receptors. Equivalent results were obtained for identical phosphorylation inhibition experiments for the parent VEGF trap, uncleaved VEGF trap, and cleaved VEGF mini-trap. The results are shown in Table 1.

TABLE 1

Trap	Kinetic Dissociation Rate (1/s)	T _{1/2} (hr)
parent VEGF trap	$5.51 \times 10^{-5} \pm 0.94\%$	3.5
uncleaved VEGF trap	$4.93 \times 10^{-5} \pm 0.70\%$	3.9
cleaved VEGF mini-trap	$5.46 \times 10^{-5} \pm 0.62\%$	3.53
R1R2-myc myc his monomer	$6.74 \times 10^{-3} \pm 0.38\%$	0.028

Example 3. Construction of Plasmids Encoding VEGF Mini-Traps

[0080] VEGF mini-traps were constructed from a precursor of the parent VEGF trap, VEGFR1R2.FcΔC1(a) (SEQ ID NOs:9-10), in which the three amino acids glycine-alanine-proline served as a linker between the Flk1 D3 and FcΔC1(a). This plasmid, pTE115 was used in the construction of the VEGF mini-traps because the linker DNA sequence included a Srf I restriction endonuclease recognition sequence that facilitated engineering the VEGF trap. In all other respects, the VEGF trap encoded by pTE115 is identical to that of the VEGF trap, VEGFR1R2.FcΔC1(a) (SEQ ID NOs:9-10) described in detail in PCT publication WO/0075319.

[0081] Two VEGF mini-traps were constructed with multimerization domains consisting of either a single cysteine residue (R1R2_C) (SEQ ID NO:2) or the amino acids ACGC (SEQ ID NO:4) (R1R2_{ACGC}) (SEQ ID NO:5) added to the C-terminus of receptor components Flt1D2.Flk1D3. Both of these constructs are capable of forming homo-dimeric molecules stabilized by one (R1R2_C) or two (R1R2_{ACGC}) intermolecular disulfides.

[0082] The plasmid pTE517 was made by removing the 690 bp fragment generated by digestion of pTE115 DNA with Srf I and Not I and inserting the synthetic DNA fragment formed by annealing the oligos R1R2NC (SEQ ID NO:14) and R1R2CC (SEQ ID NO:15). The resulting plasmid encodes R1R2_C, which consists of the Flt1D2.Flk1D3 domains followed by a cysteine residue (SEQ ID NO:23). Similarly, the plasmid pTE518 was made by removing the 690 bp fragment generated by digestion of pTE115 DNA with Srf I and Not I, followed by ligation with the synthetic DNA fragment formed by annealing the oligos R1R2NACGC (SEQ ID NO:16) and R1R2CACGC (SEQ ID NO:17). The resulting plasmid encodes R1R2_{ACGC}, which consists of the Flt1D2.Flk1D3 domains followed by the amino acids ACGC (SEQ ID NO:25).

[0083] Plasmids were also constructed to direct the expression of these mini-traps in *E. coli*. The primers R1R2N-Nco1 (SEQ ID NO:18) and R1R2CNot1 (SEQ ID NO:19) were used to amplify a DNA fragment from pTE115 that encodes amino acids G30 to K231, relative to the parental VEGF trap (SEQ ID NO:10). Amplification of this sequence resulted in fusion of an initiating methionine

codon at the 5' end and fusion of the codon for cysteine, followed by a stop codon, at the 3' end (SEQ ID NO:2). This DNA fragment was then cloned into the Nco I and Not I sites of the *E. coli* expression plasmid pRG663 to yield pRG1102 such that expression of R1R2_C was dependent on transcription from the phage T7 Φ 1.1 promoter. Induction of gene expression from pRG1102 results in accumulation of R1R2_{Cys} in the cytoplasm of the *E. coli* host strain RFJ238. Similarly, the primers R1R2N-Nco1 (SEQ ID NO:18) and R1R2ACGC-N ot1 (SEQ ID NO:20) were used to amplify a DNA fragment from pTE115 that encodes amino acids G30 to K231 (SEQ ID NO:10) resulting in fusion of an initiating methionine codon at the 5' end and fusion of codons for ACGC (SEQ ID NO:4), followed by a stop codon, at the 3' end (SEQ ID NO:5). This fragment was then cloned into the Nco I and Not I sites of the *E. coli* expression plasmid pRG663 to yield pRG1103 such that expression of R1R2_{ACGC} was dependent on transcription from the phage T7 Φ 1.1 promoter. Induction of gene expression from both pRG1102 and pRG1103 resulted in accumulation of R1R2_C or R1R2_{ACGC}, respectively, in the cytoplasm of the *E. coli* host strain RFJ238.

Example 4. Purification and characterization of VEGF mini-traps from *E. coli*

[0084] Both R1R2_C and R1R2_{ACGC} were expressed as cytoplasmic proteins in *E. coli* and were purified by the same method. Induction of the phage T7 Φ 1.1 promoter on either pRG1102 or pRG1103 in the *E. coli* K12 strain RFJ238 resulted in accumulation of the protein in the cytoplasm. After induction, cells were collected by centrifugation, resuspended in 50 mM Tris-HCl, pH 7.5, 20 mM EDTA, and lysed by passage through a Niro-Soavi cell homogenizer. Inclusion bodies were collected from lysed cells by centrifugation, washed once in distilled H₂O, then solubilized in 8 M guanidinium-HCl, 50 mM Tris-HCl, pH 8.5, 100 mM sodium sulfite, 10 mM sodium tetrathionate and incubated at room temperature for 16 hours. Clarified supernatant was fractionated on an S300 column equilibrated with 6 M guanidinium-HCl, 50 mM Tris-HCl, pH 7.5. Fractions containing R1R2_C were pooled and dialyzed against 6M Urea, 50 mM Tris-HCl, pH 7.5. Dialyzed protein was diluted to 2M Urea, 50 mM Tris-HCl, pH 8.5, 2 mM cysteine then stirred slowly for 7 days at 4°C. Refolded protein was dialyzed against 50 mM Tris-HCl, pH 7.5 then loaded onto an SP-sepharose column equilibrated with 50 mM Tris-HCl, pH 7.5 and eluted with a NaCl gradient from 0 to 1 M in 50 mM Tris-HCl, pH 7.5. Fractions containing R1R2_C were pooled, concentrated, and loaded onto a Superdex 200 column equilibrated with 50 mM Tris-HCl, pH 7.5, 150 mM NaCl. Fractions containing mini-trap dimer were collected and pooled. The molecular weight of purified mini-trap was estimated to be about 46 kD by SDS-PAGE.

[0085] BIAcore assays were conducted (as described in WO/0075319) to determine trap affinity for VEGF, and the results showed that the R1R2_C and R1R2_{ACGC} mini-traps had VEGF affinity comparable to the full length VEGF trap (Table 2).

TABLE 2

Trap	Kinetic Dissociation Rate (1/s)	T _{1/2} (hr)
VEGF trap	4.23 x 10 ⁻⁵	4.53
R1R2 _C	3.39 x 10 ⁻⁵	5.68
R1R2 _{ACGC}	3.41 x 10 ⁻⁵	5.65

Example 5. Expression of VEGF mini-traps in CHO K1

[0086] Expression of the VEGF mini-traps encoded by pTE517 and pTE518 is dependent on transcription from the human CMV-MIE promoter and results in secretion of the mini-traps into the culture medium when expressed in CHO cells. When expressed as secreted proteins in CHO K1, both mini-traps were found in the conditioned media and estimation of their molecular weight by SDS-PAGE suggested, as expected, that the proteins were glycosylated. Analysis by SDS-PAGE also indicated that the mini-traps were capable of forming homo-dimeric molecules stabilized by intermolecular disulfide(s) between the C-terminal cysteine(s). Specifically, the R1R2_C mini-trap efficiently formed covalent dimers when expressed as a secreted protein in CHO cells.

Example 6. Construction and expression of a single chain VEGF mini-trap

[0087] A VEGF mini-trap was also constructed that did not require a multimerization domain (SEQ ID NO:24). This mini-trap was constructed by direct fusion of one Flt1D2.Flk1D3 domain (R1R2) (amino acids 30-231 of SEQ ID NO:24) to a second Flt1D2.Flk1D3 domain (R1R2) (amino acids 234-435 of SEQ ID NO:24) with a Gly-Pro linker between the tandem receptor domains (amino acids 232-233 of SEQ ID NO:24).

[0088] To construct a gene encoding tandem Flt1D2.Flk1D3 domains, a DNA fragment was synthesized (Blue Heron Biotechnology) that encoded one Flt1D2.Flk1D3 domain that minimized DNA homology with the Flt1D2.Flk1D3 domain-encoding DNA found in pTE115. This synthetic DNA fragment was cloned as a Srf I-Not I fragment into the Srf I-Not I sites of pTE115 to yield pTE570, which expresses the R1R2-R1R2 VEGF mini-trap from the CMV-MIE promoter. When this plasmid is transfected into CHO K1 cells the R1R2-R1R2 VEGF mini-trap accumulates in the culture medium.

Example 7. Construction and expression of a VEGF mini-trap

[0089] A VEGF mini-trap was constructed as described above, by direct fusion of one Flt1D2.Flk1D3 domain (R1R2) (amino acids 30-231 of SEQ ID NO:26) with a C-terminal nine amino acid sequence terminating in CPPC. When this plasmid is transfected into CHO K1 cells the VEGF mini-trap of SEQ ID NO:26 is secreted into the culture medium. Subsequent purification by non-reducing SDS-PAGE electrophoresis as well as native light-scattering analysis identified a trap molecule with molecular weight approximately 64 kDa. This molecular weight indicates that a covalent dimer was formed between two fusion polypeptides of SEQ ID NO:26. Similar experiments were conducted with plasmids encoding the fusion polypeptides of SEQ ID NOS:27 and 28, and similarly showed these molecules formed homodimeric traps. Affinity determinations for human VEGF-165 binding to EGF traps composed of dimers of SEQ ID NO:10 and SEQ ID NO:26 are shown in Table 3.

TABLE 3

VEGF Trap	k_a (1/Ms)	k_d (1/s)	KD (M)
SEQ ID NO:10	$2.73 \times 10^{+7}$	1.79×10^{-5}	6.55×10^{-13}
SEQ ID NO:26	$2.00 \times 10^{+7}$	6.56×10^{-6}	3.28×10^{-13}
SEQ ID NO:26	$2.61 \times 10^{+7}$	5.77×10^{-6}	2.21×10^{-13}

We claim:

1. An isolated nucleic acid molecule encoding a fusion polypeptide consisting of components $(R1R2)_X$ or $(R1R3)_Y$, and a fusion partner (FP), wherein $X \geq 1$, $Y \geq 1$, R1 is vascular endothelial cell growth factor (VEGF) receptor component Ig domain 2 of Flt-1 and R2 is Ig domain 3 of Flk-1, R3 is Ig domain 3 of Flt-4.
2. The isolated nucleic acid of claim 1, wherein the fusion partner (FP) is a multimerizing component (MC) capable of interacting with another MC to form a multimeric structure.
3. The isolated nucleic acid of claim 3, wherein the MC is selected from the group consisting of (i) a multimerizing component comprising a cleavable region (C-region), (ii) a truncated multimerizing component, (iii) an amino acid sequence between 1 to about 200 amino acids in length having at least one cysteine residue, (iv) a leucine zipper, (v) a helix loop motif, (vi) a coil-coil motif, and (vii) an immunoglobulin domain.
4. A fusion polypeptide encoded by the nucleic acid molecule of claims 1 to 3.
5. The fusion polypeptide of claim 4, having the amino acid sequence of SEQ ID NO:26, 27, or 28.
6. A replicable expression vector capable in a transformed host cell comprising the nucleic acid molecule of claims 1 to 3.
7. A method of producing a VEGF fusion polypeptide, comprising the steps of introducing into a suitable expression system the expression vector of claim 6, and effecting expression of the VEGF fusion polypeptide.
8. A vascular endothelial cell growth factor (VEGF) trap, comprising a multimer of two or more fusion polypeptides of claim 4.
9. The VEGF trap of claim 8, which is a dimer.
10. A dimeric VEGF trap comprising two fusion polypeptides comprising the amino acid sequence of SEQ ID NO:26, 27, or 28.
11. A pharmaceutical composition comprising the fusion polypeptide of claims 8 or 9, and a pharmaceutically acceptable carrier.

12. A method of treating a disease or condition which is improved, ameliorated, or inhibited by removal or inhibition of vascular endothelial growth factor (VEGF), comprising administering the pharmaceutical composition of claim 11 to a subject in need thereof.
13. The method of claim 12, wherein the disease or condition is an ocular disease or condition.
14. The method of claim 13, wherein the ocular disease or condition is age related macular degeneration.
15. An isolated nucleic acid molecule encoding a fusion polypeptide consisting of receptor components (R1R2)_X or (R1R3)_Y, and a multimerizing component (MC) capable of interacting with another MC to form a multimeric structure, wherein $X \geq 1$, $Y \geq 1$, R1 is vascular endothelial cell growth factor (VEGF) receptor component Ig domain 2 of Flt-1 and R2 is Ig domain 3 of Flk-1, R3 is Ig domain 3 of Flt-4, wherein the multimerizing component (MC) is selected from the group consisting of (i) a MC comprising a cleavable region (C-region), (ii) a truncated MC, (iii) an amino acid sequence between 1 to about 200 amino acids in length having at least one cysteine residue, (iv) a leucine zipper, (v) a helix loop motif, (vi) a coil-coil motif, and (vii) an immunoglobulin domain.
16. The isolated nucleic acid molecule of claim 15, wherein the receptor components are (R1R2)_X and the multimerizing component is an amino acid sequence between 1 to about 200 amino acids in length having at least one cysteine residue.
17. The isolated nucleic acid molecule of claim 16, wherein the receptor component is R1R2, X is 1, and the multimerizing component is an amino acid sequence 1-15 amino acids in length with 1-2 cysteine residues.
18. A fusion polypeptide capable of binding vascular endothelial growth factor (VEGF) encoded by the nucleic acid molecule of claims 15 to 17.
19. The fusion polypeptide of claim 18, comprising the amino acid sequence of SEQ ID NO:26, 27 or 28.
20. A fusion polypeptide consisting of receptor components (R1R2)_X or (R1R3)_Y, and a multimerizing component (MC) capable of interacting with another MC to form a multimeric structure, wherein $X \geq 1$, $Y \geq 1$, R1 is vascular endothelial cell growth factor (VEGF) receptor component Ig domain 2 of Flt-1 and R2 is Ig domain 3 of Flk-1, R3 is Ig domain 3 of Flt-4, wherein the multimerizing component (MC) is selected from the group consisting of (i) a MC comprising a cleavable region (C-region), (ii) a truncated MC, (iii) an amino acid sequence between 1 to about 200 amino acids in length having at least one cysteine residue, (iv) a leucine zipper, (v) a helix loop motif, (vi) a coil-coil motif, and (vii) an immunoglobulin domain.

21. The fusion polypeptide of claim 20, wherein the receptor components are $(R1R2)_X$ and the multimerizing component is an amino acid sequence between 1 to about 200 amino acids in length having at least one cysteine residue.

22. The fusion polypeptide of claim 21, wherein the receptor component is $R1R2$, X is 1, and the multimerizing component is an amino acid sequence 1-15 amino acids in length with 1-2 cysteine residues.

23. A dimeric VEGF trap composed of two of the fusion polypeptides of claims 20 to 22.

24. An article of manufacturing comprising:

(a) packaging material; and

(b) a pharmaceutical agent contained within said packaging material;

wherein the pharmaceutical agent comprises at least one VEGF trap consisting of receptor components $(R1R2)_X$ or $(R1R3)_Y$, and a multimerizing component (MC) capable of interacting with another MC to form a multimeric structure, wherein $X \geq 1$, $Y \geq 1$, and wherein the packaging material comprises a label or package insert which indicates that said VEGF-specific fusion polypeptide can be used for treating a VEGF-mediated disease or condition.

SEQUENCE LISTING

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Fandl, James P.
Papadopoulos, Nicholas J.

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Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser
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Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser
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Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser
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Phe	Val	Glu	Met	Tyr	Ser	Glu	Ile	Pro	Glu	Ile	Ile	His	Met	Thr	Glu	35	40	45	
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Tyr	Lys	Glu	Ile	Gly	Leu	Leu	Thr	Cys	Glu	Ala	Thr	Val	Asn	Gly	His	100	105	110	
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Lys	Leu	Val	Leu	Asn	Cys	Thr	Ala	Arg	Thr	Glu	Leu	Asn	Val	Gly	Ile	145	150	155	160
Asp	Phe	Asn	Trp	Glu	Tyr	Pro	Ser	Ser	Lys	His	Gln	His	Lys	Lys	Leu	165	170	175	
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Leu	Ser	Thr	Leu	Thr	Ile	Asp	Gly	Val	Thr	Arg	Ser	Asp	Gln	Gly	Leu	195	200	205	
Tyr	Thr	Cys	Ala	Ala	Ser	Ser	Gly	Leu	Met	Thr	Lys	Lys	Asn	Ser	Thr	210	215	220	
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      65          70          75          80
Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
      85          90          95
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
      100          105          110
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      115          120          125
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Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
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                      35                      40                      45
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Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys
65                      70                      75                      80
Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
                      85                      90                      95
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
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Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile
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Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
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 Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
 85 90 95
 Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
 100 105 110
 Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile
 115 120 125
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 Glu Leu Ser Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Application of: FURFINE et al.

Confirmation No.: TBA

Application No.: TBA

Art Unit: TBA

Filed: 09 July 2010

Examiner: TBA

For: VEGF Antagonist Formulations for
Intravitreal Administration

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

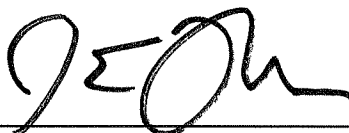
STATEMENT VERIFYING SEQUENCE LISTING UNDER § 1.821(e)

Dear Sir:

Applicants submit herewith a paper copy and a computer readable form of the identical sequence listing, which is incorporated in its entirety into the specification by reference. The sequence listing information recorded in computer readable form is identical to the written (PDF) sequence listing, as specified under 37 CFR § 1.821(e) and (f).

Respectfully submitted,

By



Name: Joseph ZAHNER, Reg. No. 48,224

Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, NY 10591
Tel.: 973-593-1595

SCORE Placeholder Sheet for IFW Content

Application Number: 12833417

Document Date: 07/09/2010

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- **Sequence Listing**

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

To access the documents in the SCORE database, refer to instructions developed by SIRA.

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- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (<http://es/ScoreAccessWeb/>).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: February 8, 2006

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Sequence Listing was accepted.

If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).

Reviewer: Anne Corrigan

Timestamp: [year=2011; month=6; day=24; hr=10; min=43; sec=38; ms=481;]

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Application No: 12833417 Version No: 1.0

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 Dix, Daniel
 Graham, Kenneth
 Frye, Kelly

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Met Tyr Ser Glu Ile Pro Glu Ile His Met Thr Glu Gly Arg Glu
 35          40          45
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu
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Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
 65          70          75          80
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu
 85          90          95
Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys
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Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val
115          120          125
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Electronic Acknowledgement Receipt

Adjustment date: 06/29/2011 MTEKLEMI
 07/12/2010 INTEFSW 00001646 180650 12833417
 04 FC:1201 1100.00 CR

EFS ID:	7985892
Application Number:	12833417
International Application Number:	
Confirmation Number:	6438
Title of Invention:	VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION
First Named Inventor/Applicant Name:	Eric FURFINE
Customer Number:	26693
Filer:	Joseph Zahner
Filer Authorized By:	
Attorney Docket Number:	5050C
Receipt Date:	09-JUL-2010
Filing Date:	
Time Stamp:	15:25:53
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$2190
RAM confirmation Number	1646
Deposit Account	180650
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	



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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	TOT CLAIMS	IND CLAIMS
12/833,417	07/09/2010	1614	1090	5050C	19	2

CONFIRMATION NO. 6438

26693
REGENERON PHARMACEUTICALS, INC
777 OLD SAW MILL RIVER ROAD
TARRYTOWN, NY 10591

FILING RECEIPT



Date Mailed: 07/12/2011

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

Eric FURFINE, Concord, MA;
Daniel DIX, Lagrangeville, NY;
Kenneth S. GRAHAM, Pleasant Valley, NY;
Kelly FRYE, Pomona, NY;

Assignment For Published Patent Application

Regeneron Pharmaceuticals, Inc., Tarrytown, NY

Power of Attorney:

Valeta Gregg-Emery--35127
Tor Smeland--43131
Ying - Zi Yang--52381

Domestic Priority data as claimed by applicant

This application is a CON of 12/560,885 09/16/2009 PAT 7,807,164
which is a DIV of 11/818,463 06/14/2007 PAT 7,608,261
which claims benefit of 60/814,484 06/16/2006

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see <http://www.uspto.gov> for more information.)

If Required, Foreign Filing License Granted: 06/28/2011

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 12/833,417**

Projected Publication Date: 10/20/2011

Non-Publication Request: No

Early Publication Request: No
Title

VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION

Preliminary Class

514

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Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

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Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

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For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, <http://www.stopfakes.gov>. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 12/833,417	
APPLICATION AS FILED - PART I							
(Column 1)		(Column 2)		SMALL ENTITY		OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE(\$)	FEE(\$)		RATE(\$)	FEE(\$)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	330
SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A	N/A			N/A	540
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	220
TOTAL CLAIMS (37 CFR 1.16(j))	19	minus 20 = *			OR	x 52 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2	minus 3 = *				x 220 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s)) If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$270 (\$135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).							0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
			TOTAL			TOTAL	1090
* If the difference in column 1 is less than zero, enter "0" in column 2.							
APPLICATION AS AMENDED - PART II							
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY	
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)
	Total (37 CFR 1.16(i))	*	Minus **	=	x =	OR	x =
	Independent (37 CFR 1.16(h))	*	Minus ***	=	x =	OR	x =
	Application Size Fee (37 CFR 1.16(s))					OR	
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					OR	
			TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY	
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)
	Total (37 CFR 1.16(i))	*	Minus **	=	x =	OR	x =
	Independent (37 CFR 1.16(h))	*	Minus ***	=	x =	OR	x =
	Application Size Fee (37 CFR 1.16(s))					OR	
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					OR	
			TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3. ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.							



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NOTICE OF ALLOWANCE AND FEE(S) DUE

26693 7590 09/16/2011
REGENERON PHARMACEUTICALS, INC
777 OLD SAW MILL RIVER ROAD
TARRYTOWN, NY 10591

EXAMINER

LOCKARD, JON MCCLELLAND

ART UNIT

PAPER NUMBER

1647

DATE MAILED: 09/16/2011

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
-----------------	-------------	----------------------	---------------------	------------------

12/833,417

07/09/2010

Eric FURFINE

5050C

6438

TITLE OF INVENTION: VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	\$1510	\$300	\$0	\$1810	12/16/2011

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

**Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

26693 7590 09/16/2011
REGENERON PHARMACEUTICALS, INC
777 OLD SAW MILL RIVER ROAD
TARRYTOWN, NY 10591

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
-----------------	-------------	----------------------	---------------------	------------------

12/833,417	07/09/2010	Eric FURFINE	5050C	6438
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TITLE OF INVENTION: VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
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nonprovisional	NO	\$1510	\$300	\$0	\$1810	12/16/2011
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EXAMINER	ART UNIT	CLASS-SUBCLASS
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LOCKARD, JON MCCLELLAND	1647	424-134100
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1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

- (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, 1 _____
- (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. 2 _____
- 3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

- ☐ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s); (Please first reapply any previously paid issue fee shown above)

- ☐ A check is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. Change in Entity Status (from status indicated above)

- ☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27. ☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/833,417	07/09/2010	Eric FURFINE	5050C	6438

26693	7590	09/16/2011
REGENERON PHARMACEUTICALS, INC		
777 OLD SAW MILL RIVER ROAD		
TARRYTOWN, NY 10591		

EXAMINER
LOCKARD, JON MCCLELLAND

ART UNIT	PAPER NUMBER
1647	

DATE MAILED: 09/16/2011

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 7 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 7 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No.	Applicant(s)	
	12/833,417	FURFINE ET AL.	
	Examiner	Art Unit	
	JON M. LOCKARD	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to the submission filed 09 July 2010.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are 1-19.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 - a) ☐ All b) ☐ Some* c) ☐ None of the:
 1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: ____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.

THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
 6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
 - (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
 - 1) ☐ hereto or 2) ☐ to Paper No./Mail Date ____.
 - (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date ____.
- Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**
7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

- | | |
|--|---|
| <ol style="list-style-type: none"> 1. ☐ Notice of References Cited (PTO-892) 2. ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948) 3. ☒ Information Disclosure Statements (PTO/SB/08),
Paper No./Mail Date <u>7/9/10</u> 4. ☐ Examiner's Comment Regarding Requirement for Deposit
of Biological Material | <ol style="list-style-type: none"> 5. ☐ Notice of Informal Patent Application 6. ☐ Interview Summary (PTO-413),
Paper No./Mail Date ____. 7. ☒ Examiner's Amendment/Comment 8. ☒ Examiner's Statement of Reasons for Allowance 9. ☐ Other ____. |
|--|---|

/J. M. L./
Examiner, Art Unit 1647

/Christine J Saoud/
Primary Examiner, Art Unit 1647

Art Unit: 1647

EXAMINER'S AMENDMENT

1. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it **MUST** be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Joseph Zahner on 08 September 2011.

The application has been amended as follows:

2. In claim 1, the phrase "antagonist of SEQ ID NO:4" has been deleted, and the phrase --antagonist comprising amino acids 27-475 of SEQ ID NO:4-- inserted therefor.
3. In claim 16, the phrase "antagonist of SEQ ID NO:4" has been deleted, and the phrase --antagonist comprising amino acids 27-475 of SEQ ID NO:4-- inserted therefor.
4. In claim 19, the phrase "in at least about" has been deleted, and the phrase --in about-- inserted therefor.

Reasons for Allowance

5. The following is an examiner's statement of reasons for allowance: The closest prior art is Davis-Smyth et al. (U.S. Pat. No. 6,897,294, priority to 07 May 1996; cited by Applicant). The '294 patent teaches fusion proteins comprising an immunoglobulin constant region, the Ig domain 2 of Flt1, and the Ig domain 3 of Flk1. The '294 patent does not teach a chimeric receptor with less than all seven Ig-like domains that bind VEGF, or truncated fusion receptors

Art Unit: 1647

with less than three Ig-like domains that bind VEGF, and thus does not anticipate or render obvious the claimed invention.

6. Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Art Unit: 1647

Advisory Information

Any inquiry concerning this communication or earlier communications from the examiner should be directed to **Jon M. Lockard** whose telephone number is **(571) 272-2717**. The examiner can normally be reached on Monday through Friday, 8:00 AM to 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Jeffrey Stucker**, can be reached on **(571) 272-0911**. The fax number for the organization where this application or proceeding is assigned is **571-273-8300**.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at **866-217-9197** (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/J. M. L./
Examiner, Art Unit 1647
September 9, 2011

/Christine J Saoud/
Primary Examiner, Art Unit 1647

Search Notes 	Application/Control No. 12833417	Applicant(s)/Patent Under Reexamination FURFINE ET AL.
	Examiner JON M LOCKARD	Art Unit 1647

SEARCHED			
Class	Subclass	Date	Examiner
NONE		9/9/2011	JML

SEARCH NOTES		
Search Notes	Date	Examiner
STIC Search of SEQ ID NO:4. See sequence search results in SCORE.	9/9/2011	JML
EAST (USPAT, US-PGPUB, EPO, DERWENT): See attached search history.	9/9/2011	JML
STN (MEDLINE, SCISEARCH, EMBASE, BIOSIS): See attached search history.	9/9/2011	JML
PALM: Inventor search.	9/9/2011	JML

INTERFERENCE SEARCH			
Class	Subclass	Date	Examiner
	STIC Search of SEQ ID NO:4. See sequence search results in SCORE.	9/9/2011	JML
	EAST (USPAT, UPAD): See attached search history.	9/9/2011	JML
	PALM: Inventor search.	9/9/2011	JML

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Receipt date: 07/09/2010

Doc code: IDS

Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)

Approved for use through 07/31/2012. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		TBA 12/833,417	
	Filing Date		2010-07-09	
	First Named Inventor	Eric FURFINE		
	Art Unit	TBA 1647		
	Examiner Name	TBA Jon Lockard		
	Attorney Docket Number	5050C		

U.S.PATENTS						
Examiner Initial*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
/J.L./ 	1	6100071	A	2000-08-08	Davis-Smyth et al.	
	2	6897294	B2	2005-05-24	Davis-Smyth et al.	
	3	7052691	B2	2006-05-30	Sleeman et al.	

If you wish to add additional U.S. Patent citation information please click the Add button.

U.S.PATENT APPLICATION PUBLICATIONS						
Examiner Initial*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
/J.L./	1	20060217311	A1	2006-09-28	DIX et al.	
/J.L./	2	20050281831	A1	2005-12-22	Davis-Smyth et al.	

If you wish to add additional U.S. Published Application citation information please click the Add button.

FOREIGN PATENT DOCUMENTS								
Examiner Initial*	Cite No	Foreign Document Number ³	Country Code ²	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T ⁵

Receipt date: 07/09/2010

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number			
	Filing Date		2010-07-09	
	First Named Inventor	Eric FURFINE		
	Art Unit	TBA		
	Examiner Name	TBA		
	Attorney Docket Number	5050C		

/J.L./ ↓	1	WO 2006/047325			2006-05-04	Genentech, Inc.		☐
	2	WO 2005/000895			2005-01-06	Regeneron Phrma, Inc.		☐
/J.L./ ↓	3	WO 2006/104852			2006-10-05	Regeneron Phrma, Inc.		☐

If you wish to add additional Foreign Patent Document citation information please click the Add button

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	1		☐

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature	/Jon Lockard/	Date Considered	09/09/2011
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

12/833,417

/JML/

* * * * * STN Columbus * * * * *

=> DIS HIST

(FILE 'HOME' ENTERED AT 16:29:28 ON 09 SEP 2011)

FILE 'MEDLINE, SCISEARCH, EMBASE, BIOSIS' ENTERED AT 16:33:05 ON 09 SEP 2011

L1	2833 S VEGF (S) (ANTAGONIST OR TRAP)
L2	0 S VEGFR1R2
L3	59 S L1 AND OPHTHALMIC
L4	0 S L2 AND FORMULATION
L5	14 S L1 AND FORMULATION
L6	71 S L3 OR L5
L7	62 DUP REM L6 (9 DUPLICATES REMOVED)



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 Alexandria, Virginia 22313-1450
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BIB DATA SHEET

CONFIRMATION NO. 6438

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.		
12/833,417	07/09/2010	424	1647	5050C		
RULE						
APPLICANTS Eric FURFINE, Concord, MA; Daniel DIX, Lagrangeville, NY; Kenneth S. GRAHAM, Pleasant Valley, NY; Kelly FRYE, Pomona, NY;						
** CONTINUING DATA ***** This application is a CON of 12/560,885 09/16/2009 PAT 7,807,164 which is a DIV of 11/818,463 06/14/2007 PAT 7,608,261 which claims benefit of 60/814,484 06/16/2006						
** FOREIGN APPLICATIONS *****						
** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ** 06/28/2011						
Foreign Priority claimed	☐ Yes ☒ No	☐ Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met	☐ Yes ☐ No		MA	0	19	2
Verified and	/JON MCCLELLAND LOCKARD/ Examiner's Signature		Initials			
Acknowledged						
ADDRESS REGENERON PHARMACEUTICALS, INC 777 OLD SAW MILL RIVER ROAD TARRYTOWN, NY 10591 UNITED STATES						
TITLE VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION						
FILING FEE RECEIVED 1090	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:			☐ All Fees		
				☐ 1.16 Fees (Filing)		
				☐ 1.17 Fees (Processing Ext. of time)		
				☐ 1.18 Fees (Issue)		
				☐ Other _____		
				☐ Credit		

Issue Classification 	Application/Control No. 12833417	Applicant(s)/Patent Under Reexamination FURFINE ET AL.
	Examiner JON M LOCKARD	Art Unit 1647

ORIGINAL						INTERNATIONAL CLASSIFICATION												
CLASS		SUBCLASS				CLAIMED					NON-CLAIMED							
424		134.1				A	6	1	K	38 / 18 (2006.01.01)								
CROSS REFERENCE(S)						C	0	7	K	14 / 71 (2006.01.01)								
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)																	
424	192.1																	
514	1.1	8.1																
530	350																	

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47															
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
	1		17												
	2		18												
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	14														
	15														
	16														

/JON M LOCKARD/ Examiner.Art Unit 1647 (Assistant Examiner)	09/09/2011 (Date)	Total Claims Allowed: 19	
/CHRISTINE J SAOUD/ Primary Examiner.Art Unit 1647 (Primary Examiner)	09/10/2011 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure NONE

EAST Search History

EAST Search History (Prior Art)

/JML/

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	16	vegfr1r2\$ and ophthalmic	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2011/09/09 15:50
L2	344	(vegf adj (trap or antagonist)) and ophthalmic	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2011/09/09 15:51
L3	40	(vegf adj (trap or antagonist)) same ophthalmic	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2011/09/09 15:52
L4	18	l3 same formulation\$	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2011/09/09 15:53
L5	695	(furfine-e\$ dix-d\$ graham-k\$ frye-k\$).in.	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2011/09/09 15:53
L6	5	l5 and vegfr1r2\$	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2011/09/09 15:53

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L7	7	vegfr1r2\$ and ophthalmic	USPAT; UPAD	OR	OFF	2011/09/09 15:54
L8	58	(vegf adj (trap or antagonist)) and ophthalmic	USPAT; UPAD	OR	OFF	2011/09/09 15:54
L9	12	(vegf adj (trap or antagonist)) same ophthalmic	USPAT; UPAD	OR	OFF	2011/09/09 15:54
L10	8	l9 same formulation\$	USPAT; UPAD	OR	OFF	2011/09/09 15:54
L11	227	(furfine-e\$ dix-d\$ graham-k\$ frye-k\$).in.	USPAT; UPAD	OR	OFF	2011/09/09 15:55
L12	1	l11 and vegfr1r2\$	USPAT; UPAD	OR	OFF	2011/09/09 15:55

9/ 9/ 2011 3:56:54 PM

C:\ Users\ jlockard\ Documents\ EAST\ Workspaces\ 12833417.wsp

Inventor Information for 12/833417

/ JML /

Inventor Name	City	State/Country
<u>FURFINE, ERIC</u>	CONCORD	MASSACHUSETTS
<u>DIX, DANIEL</u>	LAGRANGEVILLE	NEW YORK
<u>GRAHAM, KENNETH S.</u>	PLEASANT VALLEY	NEW YORK
<u>FRYE, KELLY</u>	POMONA	NEW YORK

[Appln Info](#) [Contents](#) [Petition Info](#) [Atty/Agent Info](#) [Continuity/Reexam](#) [Foreign Data](#) **Inventors** [Address](#) [Fees](#) [Post Info](#) [Pre Grant Pub](#)

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
12/833,417	07/09/2010	Eric FURFINE	5050C

CONFIRMATION NO. 6438

26693
REGENERON PHARMACEUTICALS, INC
777 OLD SAW MILL RIVER ROAD
TARRYTOWN, NY 10591

PUBLICATION NOTICE



OC000000050495478

Title: VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION

Publication No. US-2011-0257601-A1

Publication Date: 10/20/2011

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently <http://www.uspto.gov/patft/>.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently <http://pair.uspto.gov/>. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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12/833,417

07/09/2010

Eric FURFINE

5050C

6438

26693 7590 10/25/2011
REGENERON PHARMACEUTICALS, INC
777 OLD SAW MILL RIVER ROAD
TARRYTOWN, NY 10591

EXAMINER

LOCKARD, JON MCCLELLAND

ART UNIT

PAPER NUMBER

1647

MAIL DATE

DELIVERY MODE

10/25/2011

PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

SUPPLEMENTAL Notice of Allowability	Application No.	Applicant(s)	
	12/833,417	FURFINE ET AL.	
	Examiner	Art Unit	
	JON M. LOCKARD	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to the submission filed 09 July 2010.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are 1-19.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some* c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. ____.

3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: ____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.

(a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached

1) ☐ hereto or 2) ☐ to Paper No./Mail Date ____.

(b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date ____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892) 2. ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948) 3. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date ____ 4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material	5. ☐ Notice of Informal Patent Application 6. ☐ Interview Summary (PTO-413), Paper No./Mail Date ____ 7. ☒ Examiner's Amendment/Comment 8. ☐ Examiner's Statement of Reasons for Allowance 9. ☐ Other ____
--	---

/J. M. L./ Examiner, Art Unit 1647	/Christine J Saoud/ Primary Examiner, Art Unit 1647
---------------------------------------	--

SUPPLEMENTAL EXAMINER'S AMENDMENT

1. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee. Authorization for this examiner's amendment was given in a telephone interview with Joseph Zahner on 20 October 2011.

The application has been amended as follows:

2. The purpose of this Supplemental Examiner's Amendment is to clarify the instructions set forth in item number 3 in the previous Examiner's Amendment (mailed 16 September 2011). In the Examiner's Amendment mailed 16 September 2011, item number 3 stated the following: "In claim 16, the phrase "antagonist of SEQ ID NO:4" has been deleted and the phrase -- antagonist comprising amino acids 27-475 of SEQ ID NO:4-- inserted therefor". Please enter the following as a replacement:

In claim 16, the phrase "the amino acid sequence" has been deleted, and the phrase --amino acids 27-475-- inserted therefor.

Art Unit: 1647

Advisory Information

Any inquiry concerning this communication or earlier communications from the examiner should be directed to **Jon M. Lockard** whose telephone number is **(571) 272-2717**. The examiner can normally be reached on Monday through Friday, 8:00 AM to 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Jeffrey Stucker**, can be reached on **(571) 272-0911**. The fax number for the organization where this application or proceeding is assigned is **571-273-8300**.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at **866-217-9197** (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/J. M. L./
Examiner, Art Unit 1647
October 20, 2011

/Christine J Saoud/
Primary Examiner, Art Unit 1647

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: **Mail** Mail Stop ISSUE FEE
Commissioner for Patents
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INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

26693 7590 09/18/2011

REGENERON PHARMACEUTICALS, INC.
 777 OLD SAW MILL RIVER ROAD
 TARRYTOWN, NY 10591

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

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I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

Joseph E. Zahner	(Depositor's name)
/Joseph E. Zahner/	(Signature)
11/7/2011	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/833,417	07/09/2010	ERIC FURFINE	50500	6438

TITLE OF INVENTION: VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION

APPL. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	\$1510	\$300	\$1810	12/18/2011

EXAMINER	ART UNIT	CLASS-SUBCLASS
LOCKARD, JON MCCLELLAND	1647	424-134100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47, Rev 03-02 or more recent) attached. Use of a Customer Number is required.

2. For printing on the patent front page, list

- (1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
- (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 3 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. Regeneron Pharmaceuticals, Inc.
2. Joseph E. Zahner
3. --

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

Regeneron Pharmaceuticals, Inc.

(B) RESIDENCE (CITY and STATE OR COUNTRY)

Tarrytown, NY US

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are enclosed:

- ☒ Issue Fee
- ☒ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies ...

4b. Payment of Fee(s):

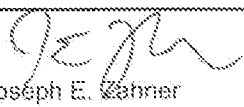
- ☐ A check in the amount of the fee(s) is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☒ The Director is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number

5. Change in Entity Status (from status indicated above)

- ☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27. ☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

The Director of the USPTO is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above. NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant, a registered attorney or agent, or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature



Date 11/7/2011

Typed or printed name

Joseph E. Zahner

Registration No. 48,224

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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Electronic Patent Application Fee Transmittal

Application Number:	12833417			
Filing Date:	09-Jul-2010			
Title of Invention:	VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION			
First Named Inventor/Applicant Name:	Eric FURFINE			
Filer:	Joseph Zahner			
Attorney Docket Number:	5050C			
Filed as Large Entity				
Utility under 35 USC 111(a) Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl issue fee	1501	1	1740	1740
Publ. Fee- early, voluntary, or normal	1504	1	300	300

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				2040

Electronic Acknowledgement Receipt

EFS ID:	11563002
Application Number:	12833417
International Application Number:	
Confirmation Number:	6438
Title of Invention:	VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION
First Named Inventor/Applicant Name:	Eric FURFINE
Customer Number:	26693
Filer:	Joseph Zahner
Filer Authorized By:	
Attorney Docket Number:	5050C
Receipt Date:	07-DEC-2011
Filing Date:	09-JUL-2010
Time Stamp:	15:00:33
Application Type:	Utility under 35 USC 111(a)

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Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 2040
RAM confirmation Number	1551
Deposit Account	180650
Authorized User	

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Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	5050C_issue_fee_1.pdf	587748	no	1
			afc3301dc085140dfc589560d943f09ba5b38425		

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	31883	no	2
			0c22ff85958b5bbc778acd534cca65cbcf9d8726		

Warnings:

Information:

Total Files Size (in bytes):			619631
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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.



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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/833,417	01/10/2012	8092803	5050C	6438

26693 7590 12/21/2011
REGENERON PHARMACEUTICALS, INC
777 OLD SAW MILL RIVER ROAD
TARRYTOWN, NY 10591

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 7 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

Eric FURFINE, Concord, MA;
Daniel DIX, Lagrangeville, NY;
Kenneth S. GRAHAM, Pleasant Valley, NY;
Kelly FRYE, Pomona, NY;

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Application of: FURFINE et al.

Confirmation No.: 6438

Application No.: 12/833,417

Art Unit: 1647

Filed: 09 July 2010

Examiner: Jon LOCKARD

For: VEGF Antagonist Formulations for
Intravitreal Administration

Attn: Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR § 1.322

Applicants respectfully request a certificate of correction for the patent granted in the above-captioned case under 37 CFR § 1.322 due to a mistake on behalf of the Office. Claims 1 and 16 of issued patent number US 8,092,803 B2 refer to "amino acids 27-475", which instead should read --amino acids 27-457--. The Office, in its Examiner's amendment of 10/21/2011, inadvertently transposed the five and the seven of 457 to read as 475. Given the nature of the SEQ ID NO:4, which spans only 458 amino acids, the incorrect denotation of "amino acids 27-475 of SEQ ID NO:4" represents an impossibility.

If any other issue remains, the Office is invited to contact undersigned Agent

Respectfully submitted,

By


Name: Joseph ZAHNER, Reg. No. 48,224

Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, NY 10591
Tel.: 973-593-1595

UNITED STATES PATENT AND TRADEMARK OFFICE CERTIFICATE OF CORRECTION

Page 1 of 1

PATENT NO. : US8092803B2

APPLICATION NO.: 12/833,417

ISSUE DATE : Jan. 10, 2012

INVENTOR(S) : Eric FURFINE, Daniel DIX, Kenneth S. GRAHAM, Kelly FRYE

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 19, claim 1, line 5, that portion of the line reading "amino acids 27-475" should read --amino acids 27-457--.

Column 20, claim 16, line 14, that portion of the line reading "amino acids 27-475" should read --amino acids 27-457--.

MAILING ADDRESS OF SENDER (Please do not use customer number below):

Joseph E. Zahner, Reg. No. 48,224, Agent for Applicant, Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591

This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	14679106
Application Number:	12833417
International Application Number:	
Confirmation Number:	6438
Title of Invention:	VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION
First Named Inventor/Applicant Name:	Eric FURFINE
Customer Number:	26693
Filer:	Joseph Zahner
Filer Authorized By:	
Attorney Docket Number:	5050C
Receipt Date:	11-JAN-2013
Filing Date:	09-JUL-2010
Time Stamp:	15:27:23
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Certificate of Correction	Cert_corr_transmittal.pdf	163073 89b4182d851f35dcfbff355abc7c53506a7df46c	no	1

Warnings:

Information:

2	Request for Certificate of Correction	5050C_certificate_of_correction.pdf	164663 b165bac13bc5836fca05433d744445d71f8d39f	no	2
Warnings:					
Information:					
Total Files Size (in bytes):				327736	
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					



UNITED STATES DEPARTMENT OF COMMERCE
COMMISSIONER FOR PATENTS
P. O. BOX 1450
ALEXANDRIA, VA 22313-1450

Date : 1/15/13
Patent No. : 8,092,803 B2
Serial No. : 12/833,417
Inventor(s) : Furfine et al.
Issue Date : January 10, 2012
Title : VEGF ANTAGONIST FORMULATIONS FOR
INTRAUTERINE ADMINISTRATION
File No. : 5050C

Re: Consideration for Certificate of Correction

Consideration has been given your request for a certificate of correction, for the above-identified patent under the provisions of Rule 1.322.

Respecting the alleged error(s) in your request, according to the last entry of the Notice of Allowance dated 10/25/2012 clearly states that, the phrase, "amino acids 27-475", should be inserted. Therefore, no correction is in order here.

In view of the foregoing, your request is hereby denied.

Further consideration will be given concerning this matter upon receipt of a request for **Reconsideration** (reconsideration should be accompanied by supporting document(s) such as, amendment, postcard receipt, 1449/892, etc.) and should be filed and directed to Certificates of Correction Branch.

Ernest G. White, LIE

571 273-3385 / Fax 571 273-3385 or ernest.white@uspto.gov

Marietta Joyce, TL (703) 756-1586

ODM/Certificate of Corrections

REGENERON PHARMACEUTICALS, INC
777 OLD SAW MILL RIVER ROAD
TARRYTOWN NY 10591

ECW

UNITED STATES PATENT AND TRADEMARK OFFICE CERTIFICATE OF CORRECTION

Page 1 of 1

PATENT NO. : US8092803B2

APPLICATION NO.: 12/833,417

ISSUE DATE : Jan. 10, 2012

INVENTOR(S) : Eric FURFINE, Daniel DIX, Kenneth S. GRAHAM, Kelly FRYE

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 19, claim 1, line 5, that portion of the line reading "amino acids 27-475" should read --amino acids 27-457--.

Column 20, claim 16, line 14, that portion of the line reading "amino acids 27-475" should read --amino acids 27-457--.

MAILING ADDRESS OF SENDER (Please do not use customer number below):

Joseph E. Zahner, Reg. No. 48,224, Agent for Applicant, Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591

This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	12833417			
Filing Date:	09-Jul-2010			
Title of Invention:	VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION			
First Named Inventor/Applicant Name:	Eric FURFINE			
Filer:	Joseph Zahner			
Attorney Docket Number:	5050C			
Filed as Large Entity				
Utility under 35 USC 111(a) Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Certificate of correction	1811	1	100	100
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
Total in USD (\$)				100

Electronic Acknowledgement Receipt

EFS ID:	15062094
Application Number:	12833417
International Application Number:	
Confirmation Number:	6438
Title of Invention:	VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION
First Named Inventor/Applicant Name:	Eric FURFINE
Customer Number:	26693
Filer:	Joseph Zahner
Filer Authorized By:	
Attorney Docket Number:	5050C
Receipt Date:	27-FEB-2013
Filing Date:	09-JUL-2010
Time Stamp:	11:36:00
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 100
RAM confirmation Number	11869
Deposit Account	180650
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	Cert_corr_transmittal_2.pdf	54661	no	1
			95a8d80b1340eb3db83c15c8a2334e9d9a1aaf04		

Warnings:

Information:

2	Request for Certificate of Correction	5050C_certificate_of_correction.pdf	164663	no	2
			b165bac13bc5836fc1a05433d74445d71f8d39f		

Warnings:

Information:

3	Fee Worksheet (SB06)	fee-info.pdf	29879	no	2
			8086a8f6aaa644e3b3554a1f1cbb78369b1a5aad		

Warnings:

Information:

Total Files Size (in bytes):

249203

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Application of: FURFINE et al.	Confirmation No.: 6438
Application No.: 12/833,417	Art Unit: 1647
Filed: 09 July 2010	Examiner: Jon LOCKARD
For: VEGF Antagonist Formulations for Intravitreal Administration	

Attn: Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR § 1.323

Applicant respectfully requests a certificate of correction for the patent granted in the above-captioned case according to 37 CFR § 1.323 due to a mistake made by Applicant. Claims 1 and 16 of issued patent number US 8,092,803 B2 refer to “amino acids 27-475”, which instead should read – -amino acids 27-457--. The Office, in its Examiner’s amendment of 10/21/2011, inadvertently transposed the five and the seven of 457 to read as 475, and Applicant inadvertently agreed to the amendment. Given the nature of the SEQ ID NO:4, which spans only 458 amino acids, the incorrect denotation of “amino acids 27-475 of SEQ ID NO:4” represents an impossibility.

The mistake is “of a clerical nature, of a typographical nature, or a mistake of minor character;” and the proposed correction does not “constitute new matter or require reexamination.” *In re Arnott*, 19 USPQ2d 1049, 1052 (Comm’r Pat. 1991). Therefore, Applicant is entitled to a Certificate of Correction. The Commissioner is authorized to deduct the required fee according to § 1.20(a) from deposit account no. 18-0650.

If any other issue remains, the Office is invited to contact undersigned Agent.

Respectfully submitted,

By /Joseph E. Zahner/

Joseph ZAHNER, Reg. No. 48,224
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, NY 10591
Tel.: 973-593-1595

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 8,092,803 B2
APPLICATION NO. : 12/833417
DATED : January 10, 2012
INVENTOR(S) : Eric Furfine et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 19, claim 1, line 5, that portion of the line reading “amino acids 27-475” should read --amino acids 27-457--.

Column 20, claim 16, line 14, that portion of the line reading “amino acids 27-475” should read --amino acids 27-457--.

Signed and Sealed this
Ninth Day of April, 2013



Teresa Stanek Rea
Acting Director of the United States Patent and Trademark Office

POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date
12/833,417	July 9, 2010

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☒ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above:

96387

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

☒ The address associated with the above-mentioned Customer Number

OR

☐ The address associated with Customer Number:

OR

Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone			Email		

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

Regeneron Pharmaceuticals, Inc.

- ☐ Inventor or Joint Inventor (title not required below)
- ☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)
- ☒ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)
- ☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature	/Frank R. Cottingham/	Date (Optional)	September 19, 2017
Name	Frank R. Cottingham		
Title	Executive Director, Assistant General Counsel, Patents, Regeneron Pharmaceuticals, Inc.		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☒ Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

STATEMENT UNDER 37 CFR 3.73(c)Applicant/Patent Owner: **REGENERON PHARMACEUTICALS, INC.**Application No./Patent No.: **8,092,803**Filed/Issue Date: **January 10, 2012**Titled: **VEGF ANTAGONIST FORMULATIONS SUITABLE FOR INTRAVITREAL ADMINISTRATION****REGENERON PHARMACEUTICALS, INC.**, a **corporation**

(Name of Assignee)

(Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):1. ☒ The assignee of the entire right, title, and interest.2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):☐ The extent (by percentage) of its ownership interest is ____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.4. ☐ The recipient, via a court proceeding or the like (e.g., bankruptcy, probate), or an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel **032616**, Frame **0301**, or for which a copy thereof is attached.B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

[Page 1 of 2]

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Karl Bozicevic, Reg. No. 28,807/
Signature

October 5, 2017
Date

Karl Bozicevic
Printed or Typed Name

28,807
Title or Registration Number

Electronic Acknowledgement Receipt

EFS ID:	30575781
Application Number:	12833417
International Application Number:	
Confirmation Number:	6438
Title of Invention:	VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION
First Named Inventor/Applicant Name:	Eric FURFINE
Customer Number:	26693
Filer:	Karl Bozicevic/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	5050C
Receipt Date:	05-OCT-2017
Filing Date:	09-JUL-2010
Time Stamp:	14:15:36
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
------------------------	----

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	5050C-US_POA.pdf	110296	no	1
			3eff51c8fee3be4e1c915b5762e0407b22a17151		

Warnings:

Information:					
2	Assignee showing of ownership per 37 CFR 3.73	REGN-024CON_2017-10-05_373_c.pdf	32211 4d82e6743a7d27c7afc229e1992738aef0c4d599	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			142507		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
12/833,417	07/09/2010	Eric FURFINE	5050C

96387
Regeneron - Bozicevic, Field & Francis
201 REDWOOD SHORES PARKWAY
SUITE 200
REDWOOD CITY, CA 94065

CONFIRMATION NO. 6438
POA ACCEPTANCE LETTER



Date Mailed: 10/16/2017

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 10/05/2017.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at (571) 272-4000 or (571) 272-4200 or 1-888-786-0101.

/vnnguyen/



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
12/833,417	07/09/2010	Eric FURFINE	5050C

26693
REGENERON PHARMACEUTICALS, INC
777 OLD SAW MILL RIVER ROAD
TARRYTOWN, NY 10591

CONFIRMATION NO. 6438
POWER OF ATTORNEY NOTICE



Date Mailed: 10/16/2017

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 10/05/2017.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

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/vnguyen/

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

☒ Practitioners associated with Customer Number: 191459

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number

Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignment documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

☒ The address associated with Customer Number: 191459

OR

☐ Firm or individual name		
Address		
City	State	Zip
Country		
Telephone	Email	

Assignee name and address:
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, NY 10591-6707

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of the practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee.

Signature /Frank R. Cottingham/

Date 13 January 2023

Name Frank R. Cottingham, Ph.D., J.D.

Telephone

Title Vice President, Associate General Counsel, Intellectual Property

This collection of information is required by 37 CFR 1.31, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public, which is to update (and by the USPTO to process) the file of a patent or reexamination proceeding. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 18 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.



ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
12/833,417

RECEIPT DATE / TIME
02/28/2023 04:20:40 PM ET

ATTORNEY DOCKET #
5050C

Title of Invention

VEGF ANTAGONIST FORMULATIONS FOR INTRAVITREAL ADMINISTRATION

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
 under 35 USC 111(a)

PATENT # 8092803

CONFIRMATION # 6438

FILED BY Kathi Moore

PATENT CENTER # 61663711

FILING DATE 07/09/2010

CUSTOMER # 96387

FIRST NAMED Eric FURFINE
INVENTOR

CORRESPONDENCE -
ADDRESS

AUTHORIZED BY Michael Lewis

Documents

TOTAL DOCUMENTS: 2

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
P35063US03-373c.pdf	3	Assignee showing of ownership per 37 CFR 3.73	110 KB
POA-Regeneron.pdf	2	Power of Attorney	3810 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
P35063US03-373c.pdf	9D19388EA4335405730FC51E324DC562EB4282BF82E123CF23 D56CBCA9425F9B70DDF26285BC422906D3096B32144394DBA 0A29858760E823EBB1CD4C283D706

POA-Regeneron.pdf

BDECE05D2F51BBCDB37E674E496644E6B6A6D74B020A14B4
FE816D8015E1EC9283171078BA0A0E1076CA395ADB4C661BA
9E9C16DA415BC049CBFF10B715A0675

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)Applicant/Patent Owner: Regeneron Pharmaceuticals, Inc.Application No./Patent No.: 8,092,803 Filed/Issue Date: January 10, 2012Titled: VEGF Antagonist Formulations for Intravitreal AdministrationRegeneron Pharmaceuticals, Inc., a corporation

(Name of Assignee)

(Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☒ The assignee of the entire right, title, and interest.
2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):
- ☐ The extent (by percentage) of its ownership interest is _____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.
- ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (e.g., bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

- A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 032616, Frame 0301, or for which a copy thereof is attached.
- B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:
1. From: _____ To: _____
- The document was recorded in the United States Patent and Trademark Office at
- Reel _____, Frame _____, or for which a copy thereof is attached.
2. From: _____ To: _____
- The document was recorded in the United States Patent and Trademark Office at
- Reel _____, Frame _____, or for which a copy thereof is attached.

[Page 1 of 2]

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
 Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
 Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
 Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
 Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☐ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Michael W. Lewis/

Signature

Michael W. Lewis

Printed or Typed Name

February 28, 2023

Date

76,829

Title or Registration Number

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12/833,417	07/09/2010	Eric FURFINE	

191459
A&P - Regeneron (Prosecution)
601 Massachusetts Ave., NW
Washington, DC 20001-3743

CONFIRMATION NO. 6438
POA ACCEPTANCE LETTER



Date Mailed: 03/02/2023

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/nrhayden/



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/nrhayden/