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Application Number: 13940370 Document Date: 07/12/2013

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Celltrion Exhibit - 1095 Pg. 001
Celltrion, Inc. v. Regeneron Pharmaceuticals, Inc.

PTO/AIA/15 (03-13)
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	725A1
First Named Inventor	YANCOPOULOS
Title	Use of a VEGF Antagonist to Treat Angiogenic ...
Express Mail Label No.	N/A

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☐ **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. ☐ **Applicant asserts small entity status.** See 37 CFR 1.27
3. ☐ **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. ☑ **Specification** [*Total Pages* 24] Both the claims and abstract must start on a new page. *(See MPEP § 608.01(a) for information on the preferred arrangement)*
5. ☑ **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 1]
6. **Inventor's Oath or Declaration** [*Total Pages* ____] *(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))*
   a. ☐ Newly executed (original or copy)
   b. ☐ A copy from a prior application (37 CFR 1.63(d))
7. ☑ **Application Data Sheet** ** See note below.* See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   ☐ Landscape Table on CD
9. **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

ADDRESS TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**

ACCOMPANYING APPLICATION PAPERS

10. ☐ **Assignment Papers** (cover sheet & document(s)) Name of Assignee ____
11. ☐ **37 CFR 3.73(c) Statement** (*when there is an assignee*) ☐ **Power of Attorney**
12. ☐ **English Translation Document** (*if applicable*)
13. ☐ **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    ☐ Copies of citations attached
14. ☐ **Preliminary Amendment**
15. ☐ **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. ☐ **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. ☐ **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. ☐ **Other:** ____

*Note: (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

☑ The address associated with Customer Number: 96387 **OR** ☐ Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/ Frank R. Cottingham /	Date	July 12, 2013
Name (Print/Type)	Frank R. Cottingham	Registration No. (Attorney/Agent)	50,437

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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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.
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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:	
Filing Date:	
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Filer:	Frank Robert Cottingham
Attorney Docket Number:	725A1

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	280	280
Utility Search Fee	1111	1	600	600
Utility Examination Fee	1311	1	720	720
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1600**

Electronic Acknowledgement Receipt

EFS ID:	16298087
Application Number:	13940370
International Application Number:	
Confirmation Number:	1055
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
Filer:	Frank Robert Cottingham
Filer Authorized By:	
Attorney Docket Number:	725A1
Receipt Date:	12-JUL-2013
Filing Date:	
Time Stamp:	11:18:49
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1600
RAM confirmation Number	9349
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		725A1_Specification.pdf	529155 66488c53637f4b7715ffe32f0b6596663f6e02cd	yes	24
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Specification		1	21	
	Claims		22	23	
	Abstract		24	24	
Warnings:					
Information:					
2	Drawings-only black and white line drawings	725A1_Figure.pdf	105393 2d582f645d0c5d17d717e589b029a39331991bdb	no	1
Warnings:					
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3	Sequence Listing	725A1_SeqList-paper.pdf	151078 5b1c06a0fa4a3bbb8b721196755d8f103238fb0e	no	3
Warnings:					
Information:					
4	Sequence Listing (Text File)	725A1_SeqList.txt	6076	no	0
Warnings:					
Information:					
5	CRF Statement Paper and CRF are the same	725A1_SeqListStatement.pdf	171883 f1b5760b5b501c526e4fd94266bc7452ce8ebb81	no	1
Warnings:					
Information:					
6	Application Data Sheet	725A1_AppDataSheet.pdf	1255991 3dbd8e1d133322201e20b1e6b08ca481c8ddef80	no	6

Warnings:					
Information:					
7	Transmittal of New Application	725A1_ApplicationTransmittal.pdf	276559 1f3a69d8d94bef563f27d2443e518e782b18cb70	no	2
Warnings:					
Information:					
8	Fee Worksheet (SB06)	fee-info.pdf	32862 1e8c487df991d67f0cd6fb4c4f879022d7ef949a	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	2528997	

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<u>National Stage of an International Application under 35 U.S.C. 371</u>
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Electronic Acknowledgement Receipt

EFS ID:	16298087
Application Number:	13940370
International Application Number:	
Confirmation Number:	1055
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
Filer:	Frank Robert Cottingham
Filer Authorized By:	
Attorney Docket Number:	725A1
Receipt Date:	12-JUL-2013
Filing Date:	
Time Stamp:	11:18:49
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1600
RAM confirmation Number	9349
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		725A1_Specification.pdf	529155 66488c53637f4b7715ffe32f0b6596663f6e02cd	yes	24
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Specification		1	21	
	Claims		22	23	
	Abstract		24	24	
Warnings:					
Information:					
2	Drawings-only black and white line drawings	725A1_Figure.pdf	105393 2d582f645d0c5d17d717e589b029a39331991bdb	no	1
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
3	Sequence Listing	725A1_SeqList-paper.pdf	151078 5b1c06a0fa4a3bbb8b721196755d8f103238fb0e	no	3
Warnings:					
Information:					
4	Sequence Listing (Text File)	725A1_SeqList.txt	6076	no	0
Warnings:					
Information:					
5	CRF Statement Paper and CRF are the same	725A1_SeqListStatement.pdf	171883 f1b5760b5b501c526e4fd94266bc7452ce8ebb81	no	1
Warnings:					
Information:					
6	Application Data Sheet	725A1_AppDataSheet.pdf	1255991 3dbd8e1d133322201e20b1e6b08ca481c8ddef80	no	6

Warnings:					
Information:					
7	Transmittal of New Application	725A1_ApplicationTransmittal.pdf	276559 1f3a69d8d94bef563f27d2443e518e782b18cb70	no	2
Warnings:					
Information:					
8	Fee Worksheet (SB06)	fee-info.pdf	32862 1e8c487df991d67f0cd6fb4c4f879022d7ef949a	no	2
Warnings:					
Information:					
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<u>New International Application Filed with the USPTO as a Receiving Office</u>
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USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of International Patent Application No. PCT/US2012/020855, filed on January 11, 2012, which claims the benefit of US Provisional Application Nos. 61/432,245, filed on January 13, 2011, 61/434,836, filed on January 21, 2011, and 61/561,957, filed on November 21, 2011, the contents of which are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

[0002] The present invention relates to the field of therapeutic treatments of eye disorders. More specifically, the invention relates to the administration of VEGF antagonists to treat eye disorders caused by or associated with angiogenesis.

BACKGROUND

[0003] Several eye disorders are associated with pathological angiogenesis. For example, the development of age-related macular degeneration (AMD) is associated with a process called choroidal neovascularization (CNV). Leakage from the CNV causes macular edema and collection of fluid beneath the macula resulting in vision loss. Diabetic macular edema (DME) is another eye disorder with an angiogenic component. DME is the most prevalent cause of moderate vision loss in patients with diabetes and is a common complication of diabetic retinopathy, a disease affecting the blood vessels of the retina. Clinically significant DME occurs when fluid leaks into the center of the macula, the light-sensitive part of the retina responsible for sharp, direct vision. Fluid in the macula can cause severe vision loss or blindness. Yet another eye disorder associated with abnormal angiogenesis is central retinal vein occlusion (CRVO). CRVO is caused by obstruction of the central retinal vein that leads to a back-up of blood and fluid in the retina. The retina can also become ischemic, resulting in the growth of new, inappropriate blood vessels that can cause further vision loss and more serious complications. Release of vascular endothelial growth factor (VEGF) contributes to increased vascular permeability in the eye and inappropriate new vessel growth. Thus, inhibiting the angiogenic-promoting properties of VEGF appears to be an effective strategy for treating angiogenic eye disorders.

[0004] FDA-approved treatments of angiogenic eye disorders such as AMD and CRVO include the administration of an anti-VEGF antibody called ranibizumab (Lucentis®, Genentech, Inc.) on a monthly basis by intravitreal injection.

[0005] Methods for treating eye disorders using VEGF antagonists are mentioned in, *e.g.*, US 7,303,746; US 7,306,799; US 7,300,563; US 7,303,748; and US 2007/0190058. Nonetheless,

there remains a need in the art for new administration regimens for angiogenic eye disorders, especially those which allow for less frequent dosing while maintaining a high level of efficacy.

BRIEF SUMMARY OF THE INVENTION

[0006] The present invention provides methods for treating angiogenic eye disorders. The methods of the invention comprise sequentially administering multiple doses of a VEGF antagonist to a patient over time. In particular, the methods of the invention comprise sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonists. The present inventors have surprisingly discovered that beneficial therapeutic effects can be achieved in patients suffering from angiogenic eye disorders by administering a VEGF antagonist to a patient at a frequency of once every 8 or more weeks, especially when such doses are preceded by about three doses administered to the patient at a frequency of about 2 to 4 weeks. Thus, according to the methods of the present invention, each secondary dose of VEGF antagonist is administered 2 to 4 weeks after the immediately preceding dose, and each tertiary dose is administered at least 8 weeks after the immediately preceding dose. An example of a dosing regimen of the present invention is shown in Figure 1. One advantage of such a dosing regimen is that, for most of the course of treatment (*i.e.*, the tertiary doses), it allows for less frequent dosing (*e.g.*, once every 8 weeks) compared to prior administration regimens for angiogenic eye disorders which require monthly administrations throughout the entire course of treatment. (*See*, *e.g.*, prescribing information for Lucentis® [ranibizumab], Genentech, Inc.).

[0007] The methods of the present invention can be used to treat any angiogenic eye disorder, including, *e.g.*, age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, corneal neovascularization, etc.

[0008] The methods of the present invention comprise administering any VEGF antagonist to the patient. In one embodiment, the VEGF antagonist comprises one or more VEGF receptor-based chimeric molecule(s), (also referred to herein as a "VEGF-Trap" or "VEGFT"). An exemplary VEGF antagonist that can be used in the context of the present invention is a multimeric VEGF-binding protein comprising two or more VEGF receptor-based chimeric molecules referred to herein as "VEGFR1R2-FcΔC1(a)" or "aflibercept."

[0009] Various administration routes are contemplated for use in the methods of the present invention, including, *e.g.*, topical administration or intraocular administration (*e.g.*, intravitreal administration).

[0010] Aflibercept (EYLEA™, Regeneron Pharmaceuticals, Inc) was approved by the FDA in November 2011, for the treatment of patients with neovascular (wet) age-related macular degeneration, with a recommended dose of 2 mg administered by intravitreal injection every 4

weeks for the first three months, followed by 2 mg administered by intravitreal injection once every 8 weeks.

[0011] Other embodiments of the present invention will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURE

[0012] Figure 1 shows an exemplary dosing regimen of the present invention. In this regimen, a single "initial dose" of VEGF antagonist ("VEGFT") is administered at the beginning of the treatment regimen (*i.e.* at "week 0"), two "secondary doses" are administered at weeks 4 and 8, respectively, and at least six "tertiary doses" are administered once every 8 weeks thereafter, *i.e.*, at weeks 16, 24, 32, 40, 48, 56, etc.).

DETAILED DESCRIPTION

[0013] Before the present invention is 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 by the appended claims.

[0014] 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. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0015] 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.

DOSING REGIMENS

[0016] The present invention provides methods for treating angiogenic eye disorders. The methods of the invention comprise sequentially administering to a patient multiple doses of a VEGF antagonist. As used herein, "sequentially administering" means that each dose of VEGF antagonist is administered to the patient at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of a VEGF

antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist.

[0017] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the VEGF antagonist. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of VEGF antagonist, but will generally differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of VEGF antagonist contained in the initial, secondary and/or tertiary doses will vary from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment.

[0018] In one exemplary embodiment of the present invention, each secondary dose is administered 2 to 4 (*e.g.*, 2, 2½, 3, 3½, or 4) weeks after the immediately preceding dose, and each tertiary dose is administered at least 8 (*e.g.*, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of VEGF antagonist which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0019] In one exemplary embodiment of the present invention, a single initial dose of a VEGF antagonist is administered to a patient on the first day of the treatment regimen (*i.e.*, at week 0), followed by two secondary doses, each administered four weeks after the immediately preceding dose (*i.e.*, at week 4 and at week 8), followed by at least 5 tertiary doses, each administered eight weeks after the immediately preceding dose (*i.e.*, at weeks 16, 24, 32, 40 and 48). The tertiary doses may continue (at intervals of 8 or more weeks) indefinitely during the course of the treatment regimen. This exemplary administration regimen is depicted graphically in Figure 1.

[0020] The methods of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of a VEGF antagonist. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0021] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 4 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered

to the patient 8 weeks after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. For example, the present invention includes methods which comprise administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by at least 5 tertiary doses of the VEGF antagonist, wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered from 8 to 12 (*e.g.*, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12) weeks after the immediately preceding dose. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

VEGF ANTAGONISTS

[0022] The methods of the present invention comprise administering to a patient a VEGF antagonist according to specified dosing regimens. As used herein, the expression "VEGF antagonist" means any molecule that blocks, reduces or interferes with the normal biological activity of VEGF.

[0023] VEGF antagonists include molecules which interfere with the interaction between VEGF and a natural VEGF receptor, *e.g.*, molecules which bind to VEGF or a VEGF receptor and prevent or otherwise hinder the interaction between VEGF and a VEGF receptor. Specific exemplary VEGF antagonists include anti-VEGF antibodies, anti-VEGF receptor antibodies, and VEGF receptor-based chimeric molecules (also referred to herein as "VEGF-Traps").

[0024] VEGF receptor-based chimeric molecules include chimeric polypeptides which comprise two or more immunoglobulin (Ig)-like domains of a VEGF receptor such as VEGFR1 (also referred to as Flt1) and/or VEGFR2 (also referred to as Flk1 or KDR), and may also contain a multimerizing domain (*e.g.*, an Fc domain which facilitates the multimerization [*e.g.*, dimerization] of two or more chimeric polypeptides). An exemplary VEGF receptor-based chimeric molecule is a molecule referred to as VEGFR1R2-FcΔC1(a) which is encoded by the nucleic acid sequence of SEQ ID NO:1. VEGFR1R2-FcΔC1(a) comprises three components: (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130 to 231 of SEQ ID NO:2; and (3) a multimerization component ("FcΔC1(a)") comprising amino acids 232 to 457 of SEQ ID NO:2 (the C-terminal amino acid of SEQ ID NO:2 [*i.e.*, K458] may or may not be included in the VEGF antagonist used in the methods of the invention; *see e.g.*, US Patent 7,396,664). Amino acids 1-26 of SEQ ID NO:2 are the signal sequence.

[0025] The VEGF antagonist used in the Examples set forth herein below is a dimeric molecule comprising two VEGFR1R2-FcΔC1(a) molecules and is referred to herein as "VEGFT." Additional

VEGF receptor-based chimeric molecules which can be used in the context of the present invention are disclosed in US 7,396,664, 7,303,746 and WO 00/75319.

ANGIOGENIC EYE DISORDERS

[0026] The methods of the present invention can be used to treat any angiogenic eye disorder. The expression "angiogenic eye disorder," as used herein, means any disease of the eye which is caused by or associated with the growth or proliferation of blood vessels or by blood vessel leakage. Non-limiting examples of angiogenic eye disorders that are treatable using the methods of the present invention include age-related macular degeneration (*e.g.*, wet AMD, exudative AMD, etc.), retinal vein occlusion (RVO), central retinal vein occlusion (CRVO; *e.g.*, macular edema following CRVO), branch retinal vein occlusion (BRVO), diabetic macular edema (DME), choroidal neovascularization (CNV; *e.g.*, myopic CNV), iris neovascularization, neovascular glaucoma, post-surgical fibrosis in glaucoma, proliferative vitreoretinopathy (PVR), optic disc neovascularization, corneal neovascularization, retinal neovascularization, vitreal neovascularization, pannus, pterygium, vascular retinopathy, and diabetic retinopathies.

PHARMACEUTICAL FORMULATIONS

[0027] The present invention includes methods in which the VEGF antagonist that is administered to the patient is contained within a pharmaceutical formulation. The pharmaceutical formulation may comprise the VEGF antagonist along with at least one inactive ingredient such as, *e.g.*, a pharmaceutically acceptable carrier. Other agents may be incorporated into the pharmaceutical composition to provide improved transfer, delivery, tolerance, and the like. 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 antibody is administered. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, Pa., 1975), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in the context of the methods of the present invention, provided that the VEGF antagonist is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Powell et al. PDA (1998) J Pharm Sci

Technol. 52:238-311 and the citations therein for additional information related to excipients and carriers well known to pharmaceutical chemists.

[0028] Pharmaceutical formulations useful for administration by injection in the context of the present invention may be prepared by dissolving, suspending or emulsifying a VEGF antagonist in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there may be employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared can be filled in an appropriate ampoule if desired.

MODES OF ADMINISTRATION

[0029] The VEGF antagonist (or pharmaceutical formulation comprising the VEGF antagonist) may be administered to the patient by any known delivery system and/or administration method. In certain embodiments, the VEGF antagonist is administered to the patient by ocular, intraocular, intravitreal or subconjunctival injection. In other embodiments, the VEGF antagonist can be administered to the patient by topical administration, *e.g.*, via eye drops or other liquid, gel, ointment or fluid which contains the VEGF antagonist and can be applied directly to the eye. Other possible routes of administration include, *e.g.*, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral.

AMOUNT OF VEGF ANTAGONIST ADMINISTERED

[0030] Each dose of VEGF antagonist administered to the patient over the course of the treatment regimen may contain the same, or substantially the same, amount of VEGF antagonist. Alternatively, the quantity of VEGF antagonist contained within the individual doses may vary over the course of the treatment regimen. For example, in certain embodiments, a first quantity of VEGF antagonist is administered in the initial dose, a second quantity of VEGF antagonist is administered in the secondary doses, and a third quantity of VEGF antagonist is administered in the tertiary doses. The present invention contemplates dosing schemes in which the quantity of VEGF antagonist contained within the individual doses increases over time (*e.g.*, each subsequent dose contains more VEGF antagonist than the last), decreases over time (*e.g.*, each subsequent dose contains less VEGF antagonist than the last), initially increases then decreases, initially decreases then increases, or remains the same throughout the course of the administration regimen.

[0031] The amount of VEGF antagonist administered to the patient in each dose is, in most cases, a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" means a dose of VEGF antagonist that results in a detectable improvement in one or more symptoms or indicia of an angiogenic eye disorder, or a dose of VEGF antagonist that inhibits, prevents, lessens, or delays the progression of an angiogenic eye disorder. In the case of an anti-VEGF antibody or a VEGF receptor-based chimeric molecule such as VEGFR1R2-FcΔC1(a), a therapeutically effective amount can be from about 0.05 mg to about 5 mg, *e.g.*, about 0.05 mg, about 0.1 mg, about 0.15 mg, about 0.2 mg, about 0.25 mg, about 0.3 mg, about 0.35 mg, about 0.4 mg, about 0.45 mg, about 0.5 mg, about 0.55 mg, about 0.6 mg, about 0.65 mg, about 0.7 mg, about 0.75 mg, about 0.8 mg, about 0.85 mg, about 0.9 mg, about 1.0 mg, about 1.05 mg, about 1.1 mg, about 1.15 mg, about 1.2 mg, about 1.25 mg, about 1.3 mg, about 1.35 mg, about 1.4 mg, about 1.45 mg, about 1.5 mg, about 1.55 mg, about 1.6 mg, about 1.65 mg, about 1.7 mg, about 1.75 mg, about 1.8 mg, about 1.85 mg, about 1.9 mg, about 2.0 mg, about 2.05 mg, about 2.1 mg, about 2.15 mg, about 2.2 mg, about 2.25 mg, about 2.3 mg, about 2.35 mg, about 2.4 mg, about 2.45 mg, about 2.5 mg, about 2.55 mg, about 2.6 mg, about 2.65 mg, about 2.7 mg, about 2.75 mg, about 2.8 mg, about 2.85 mg, about 2.9 mg, about 3.0 mg, about 3.5 mg, about 4.0 mg, about 4.5 mg, or about 5.0 mg of the antibody or receptor-based chimeric molecule.

[0032] The amount of VEGF antagonist contained within the individual doses may be expressed in terms of milligrams of antibody per kilogram of patient body weight (*i.e.*, mg/kg). For example, the VEGF antagonist may be administered to a patient at a dose of about 0.0001 to about 10 mg/kg of patient body weight.

TREATMENT POPULATION AND EFFICACY

[0033] The methods of the present invention are useful for treating angiogenic eye disorders in patients that have been diagnosed with or are at risk of being afflicted with an angiogenic eye disorder. Generally, the methods of the present invention demonstrate efficacy within 104 weeks of the initiation of the treatment regimen (with the initial dose administered at "week 0"), *e.g.*, by the end of week 16, by the end of week 24, by the end of week 32, by the end of week 40, by the end of week 48, by the end of week 56, etc. In the context of methods for treating angiogenic eye disorders such as AMD, CRVO, and DME, "efficacy" means that, from the initiation of treatment, the patient exhibits a loss of 15 or fewer letters on the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart. In certain embodiments, "efficacy" means a gain of one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or more) letters on the ETDRS chart from the time of initiation of treatment.

EXAMPLES

[0034] The following examples are 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.

[0035] The exemplary VEGF antagonist used in all Examples set forth below is a dimeric molecule having two functional VEGF binding units. Each functional binding unit is comprised of Ig domain 2 from VEGFR1 fused to Ig domain 3 from VEGFR2, which in turn is fused to the hinge region of a human IgG1 Fc domain (VEGFR1R2-FcΔC1(a); encoded by SEQ ID NO:1). This VEGF antagonist is referred to in the examples below as "VEGFT". For purposes of the following Examples, "monthly" dosing is equivalent to dosing once every four weeks.

Example 1: Phase I Clinical Trial of Intravitreally Administered VEGF Receptor-Based Chimeric Molecule (VEGFT) in Subjects with Neovascular AMD

[0036] In this Phase I study, 21 subjects with neovascular AMD received a single intravitreal (IVT) dose of VEGFT. Five groups of three subjects each received either 0.05, 0.15, 0.5, 2 or 4 mg of VEGFT, and a sixth group of six subjects received 1 mg. No serious adverse events related to the study drug, and no identifiable intraocular inflammation was reported. Preliminary results showed that, following injection of VEGFT, a rapid decrease in foveal thickness and macular volume was observed that was maintained through 6 weeks. At Day 43 across all dose groups, mean excess retinal thickness [excess retinal thickness = (retinal thickness – 179μ)] on optical coherence tomography (OCT) was reduced from 119μ to 27μ as assessed by Fast Macular Scan and from 194μ to 60μ as assessed using a single Posterior Pole scan. The mean increase in best corrected visual acuity (BCVA) was 4.75 letters, and BCVA was stable or improved in 95% of subjects. In the 2 highest dose groups (2 and 4 mg), the mean increase in BCVA was 13.5 letters, with 3 of 6 subjects demonstrating improvement of ≥ 3 lines.

Example 2: Phase II Clinical Trial of Repeated Doses of Intravitreally Administered VEGF Receptor-Based Chimeric Molecule (VEGFT) in Subjects with Neovascular AMD

[0037] This study was a double-masked, randomized study of 3 doses (0.5, 2, and 4 mg) of VEGFT tested at 4-week and/or 12-week dosing intervals. There were 5 treatment arms in this study, as follows: 1) 0.5 mg every 4 weeks, 2) 0.5 mg every 12 weeks, 3) 2 mg every 4 weeks, 4) 2 mg every 12 weeks and 5) 4 mg every 12 weeks. Subjects were dosed at a fixed interval for the

first 12 weeks, after which they were evaluated every 4 weeks for 9 months, during which additional doses were administered based on pre-specified criteria. All subjects were then followed for one year after their last dose of VEGFT. Preliminary data from a pre-planned interim analysis indicated that VEGFT met its primary endpoint of a statistically significant reduction in retinal thickness after 12 weeks compared with baseline (all groups combined, decrease of 135μ, $p < 0.0001$). Mean change from baseline in visual acuity, a key secondary endpoint of the study, also demonstrated statistically significant improvement (all groups combined, increase of 5.9 letters, $p < 0.0001$). Moreover, patients in the dose groups that received only a single dose, on average, demonstrated a decrease in excess retinal thickness ($p < 0.0001$) and an increase in visual acuity ($p = 0.012$) at 12 weeks. There were no drug-related serious adverse events, and treatment with the VEGF antagonists was generally well-tolerated. The most common adverse events were those typically associated with intravitreal injections.

Example 3: Phase I Clinical Trial of Systemically Administered VEGF Receptor-Based Chimeric Molecule (VEGFT) in Subjects with Neovascular AMD

[0038] This study was a placebo-controlled, sequential-group, dose-escalating safety, tolerability and bioeffect study of VEGFT by IV infusion in subjects with neovascular AMD. Groups of 8 subjects meeting eligibility criteria for subfoveal choroidal neovascularization (CNV) related to AMD were assigned to receive 4 IV injections of VEGFT or placebo at dose levels of 0.3, 1, or 3 mg/kg over an 8-week period.

[0039] Most adverse events that were attributed to VEGFT were mild to moderate in severity, but 2 of 5 subjects treated with 3 mg/kg experienced dose-limiting toxicity (DLT) (one with Grade 4 hypertension and one with Grade 2 proteinuria); therefore, all subjects in the 3 mg/kg dose group did not enter the study. The mean percent changes in excess retinal thickness were: -12%, -10%, -66%, and -60% for the placebo, 0.3, 1, and 3 mg/kg dose groups at day 15 (ANOVA $p< 0.02$), and -5.6%, +47.1%, and -63.3% for the placebo, 0.3, and 1 mg/kg dose groups at day 71 (ANOVA $p< 0.02$). There was a numerical improvement in BCVA in the subjects treated with VEGFT. As would be expected in such a small study, the results were not statistically significant.

Example 4: Phase III Clinical Trials of the Efficacy, Safety, and Tolerability of Repeated Doses of Intravitreal VEGFT in Subjects with Neovascular Age-Related Macular Degeneration

A. Objectives, Hypotheses and Endpoints

[0040] Two parallel Phase III clinical trials were carried out to investigate the use of VEGFT to treat patients with the neovascular form of age-related macular degeneration (Study 1 and Study 2). The primary objective of these studies was to assess the efficacy of IVT administered VEGFT

compared to ranibizumab (Lucentis®, Genentech, Inc.), in a non-inferiority paradigm, in preventing moderate vision loss in subjects with all subtypes of neovascular AMD.

[0041] The secondary objectives were (a) to assess the safety and tolerability of repeated IVT administration of VEGFT in subjects with all sub-types of neovascular AMD for periods up to 2 years; and (b) to assess the effect of repeated IVT administration of VEGFT on Vision-Related Quality of Life (QOL) in subjects with all sub-types of neovascular AMD.

[0042] The primary hypothesis of these studies was that the proportion of subjects treated with VEGFT with stable or improved BCVA (<15 letters lost) is similar to the proportion treated with ranibizumab who have stable or improved BCVA, thereby demonstrating non-inferiority.

[0043] The primary endpoint for these studies was the prevention of vision loss of greater than or equal to 15 letters on the ETDRS chart, compared to baseline, at 52 weeks. Secondary endpoints were as follows: (a) change from baseline to Week 52 in letter score on the ETDRS chart; (b) gain from baseline to Week 52 of 15 letters or more on the ETDRS chart; (c) change from baseline to Week 52 in total NEI VFQ-25 score; and (d) change from baseline to Week 52 in CNV area.

B. Study Design

[0044] For each study, subjects were randomly assigned in a 1:1:1:1 ratio to 1 of 4 dosing regimens: (1) 2 mg VEGFT administered every 4 weeks (2Q4); (2) 0.5 mg VEGFT administered every 4 weeks (0.5Q4); (3) 2 mg VEGFT administered every 4 weeks to week 8 and then every 8 weeks (with sham injection at the interim 4-week visits when study drug was not administered (2Q8); and (4) 0.5 mg ranibizumab administered every 4 weeks (RQ4). Subjects assigned to (2Q8) received the 2 mg injection every 4 weeks to week 8 and then a sham injection at interim 4-week visits (when study drug is not to be administered) during the first 52 weeks of the studies. (No sham injection were given at Week 52).

[0045] The study duration for each subject was scheduled to be 96 weeks plus the recruitment period. For the first 52 weeks (Year 1), subjects received an IVT or sham injection in the study eye every 4 weeks. (No sham injections were given at Week 52). During the second year of the study, subjects will be evaluated every 4 weeks and will receive IVT injection of study drug at intervals determined by specific dosing criteria, but at least every 12 weeks. (During the second year of the study, sham injections will not be given.) During this period, injections may be given as frequently as every 4 weeks, but no less frequently than every 12 weeks, according to the following criteria: (i) increase in central retinal thickness of ≥100 μm compared to the lowest previous value as measured by optical coherence tomography (OCT); or (ii) a loss from the best previous letter score of at least 5 ETDRS letters in conjunction with recurrent fluid as indicated by OCT; or (iii) new or persistent fluid as indicated by OCT; or (iv) new onset classic neovascularization, or new or persistent leak on fluorescein angiography (FA); or (v) new macular hemorrhage; or (vi) 12 weeks

have elapsed since the previous injection. According to the present protocol, subjects must receive an injection at least every 12 weeks.

[0046] Subjects were evaluated at 4 weeks intervals for safety and best corrected visual acuity (BCVA) using the 4 meter ETDRS protocol. Quality of Life (QOL) was evaluated using the NEI VFQ-25 questionnaire. OCT and FA examinations were conducted periodically.

[0047] Approximately 1200 subjects were enrolled, with a target enrollment of 300 subjects per treatment arm.

[0048] To be eligible for this study, subjects were required to have subfoveal choroidal neovascularization (CNV) secondary to AMD. "Subfoveal" CNV was defined as the presence of subfoveal neovascularization, documented by FA, or presence of a lesion that is juxtafoveal in location angiographically but affects the fovea. Subject eligibility was confirmed based on angiographic criteria prior to randomization.

[0049] Only one eye was designated as the study eye. For subjects who met eligibility criteria in both eyes, the eye with the worse VA was selected as the study eye. If both eyes had equal VA, the eye with the clearest lens and ocular media and least amount of subfoveal scar or geographic atrophy was selected. If there was no objective basis for selecting the study eye, factors such as ocular dominance, other ocular pathology and subject preference were considered in making the selection.

[0050] Inclusion criteria for both studies were as follows: (i) signed Informed consent; (ii) at least 50 years of age; (iii) active primary subfoveal CNV lesions secondary to AMD, including juxtafoveal lesions that affect the fovea as evidenced by FA in the study eye; (iv) CNV at least 50% of total lesion size; (v) early treatment diabetic retinopathy study (ETDRS) best-corrected visual acuity of: 20/40 to 20/320 (letter score of 73 to 25) in the study eye; (vi) willing, committed, and able to return for all clinic visits and complete all study-related procedures; and (vii) able to read, understand and willing to sign the informed consent form (or, if unable to read due to visual impairment, be read to verbatim by the person administering the informed consent or a family member).

[0051] Exclusion criteria for both studies were as follows: 1. Any prior ocular (in the study eye) or systemic treatment or surgery for neovascular AMD except dietary supplements or vitamins. 2. Any prior or concomitant therapy with another investigational agent to treat neovascular AMD in the study eye, except dietary supplements or vitamins. 3. Prior treatment with anti-VEGF agents as follows: (a) Prior treatment with anti-VEGF therapy in the study eye was not allowed; (b) Prior treatment with anti-VEGF therapy in the fellow eye with an investigational agent (not FDA approved, e.g. bevacizumab) was allowed up to 3 months prior to first dose in the study, and such treatments were not allowed during the study. Prior treatment with an approved anti-VEGF therapy in the fellow eye was allowed; (c) Prior systemic anti-VEGF therapy, investigational or FDA/Health Canada approved, was only allowed up to 3 months prior to first dose, and was not allowed during

the study. 4. Total lesion size > 12 disc areas (30.5 mm2, including blood, scars and neovascularization) as assessed by FA in the study eye. 5. Subretinal hemorrhage that is either 50% or more of the total lesion area, or if the blood is under the fovea and is 1 or more disc areas in size in the study eye. (If the blood is under the fovea, then the fovea must be surrounded 270 degrees by visible CNV.) 6. Scar or fibrosis, making up > 50% of total lesion in the study eye. 7. Scar, fibrosis, or atrophy involving the center of the fovea. 8. Presence of retinal pigment epithelial tears or rips involving the macula in the study eye. 9. History of any vitreous hemorrhage within 4 weeks prior to Visit 1 in the study eye. 10. Presence of other causes of CNV, including pathologic myopia (spherical equivalent of –8 diopters or more negative, or axial length of 25 mm or more), ocular histoplasmosis syndrome, angioid streaks, choroidal rupture, or multifocal choroiditis in the study eye. 11. History or clinical evidence of diabetic retinopathy, diabetic macular edema or any other vascular disease affecting the retina, other than AMD, in either eye. 12. Prior vitrectomy in the study eye. 13. History of retinal detachment or treatment or surgery for retinal detachment in the study eye. 14. Any history of macular hole of stage 2 and above in the study eye. 15. Any intraocular or periocular surgery within 3 months of Day 1 on the study eye, except lid surgery, which may not have taken place within 1 month of day 1, as long as it was unlikely to interfere with the injection. 16. Prior trabeculectomy or other filtration surgery in the study eye. 17. Uncontrolled glaucoma (defined as intraocular pressure greater than or equal to 25 mm Hg despite treatment with anti-glaucoma medication) in the study eye. 18. Active intraocular inflammation in either eye. 19. Active ocular or periocular infection in either eye. 20. Any ocular or periocular infection within the last 2 weeks prior to Screening in either eye. 21. Any history of uveitis in either eye. 22. Active scleritis or episcleritis in either eye. 23. Presence or history of scleromalacia in either eye. 24. Aphakia or pseudophakia with absence of posterior capsule (unless it occurred as a result of a yttrium aluminum garnet [YAG] posterior capsulotomy) in the study eye. 25. Previous therapeutic radiation in the region of the study eye. 26. History of corneal transplant or corneal dystrophy in the study eye. 27. Significant media opacities, including cataract, in the study eye which might interfere with visual acuity, assessment of safety, or fundus photography. 28. Any concurrent intraocular condition in the study eye (e.g. cataract) that, in the opinion of the investigator, could require either medical or surgical intervention during the 96 week study period. 29. Any concurrent ocular condition in the study eye which, in the opinion of the investigator, could either increase the risk to the subject beyond what is to be expected from standard procedures of intraocular injection, or which otherwise may interfere with the injection procedure or with evaluation of efficacy or safety. 30. History of other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that might affect interpretation of the results of the study or render the subject at high risk for treatment complications. 31. Participation as a subject in any clinical study

within the 12 weeks prior to Day 1. 32. Any systemic or ocular treatment with an investigational agent in the past 3 months prior to Day 1. 33. The use of long acting steroids, either systemically or intraocularly, in the 6 months prior to day 1. 34. Any history of allergy to povidone iodine. 35. Known serious allergy to the fluorescein sodium for injection in angiography. 36. Presence of any contraindications indicated in the FDA Approved label for ranibizumab (Lucentis®). 37. Females who were pregnant, breastfeeding, or of childbearing potential, unwilling to practice adequate contraception throughout the study. Adequate contraceptive measures include oral contraceptives (stable use for 2 or more cycles prior to screening); IUD; Depo-Provera®; Norplant® System implants; bilateral tubal ligation; vasectomy; condom or diaphragm plus either contraceptive sponge, foam or jelly.

[0052] Subjects were not allowed to receive any standard or investigational agents for treatment of their AMD in the study eye other than their assigned study treatment with VEGFT or ranibizumab as specified in the protocol until they completed the Completion/Early Termination visit assessments. This includes medications administered locally (e.g., IVT, topical, juxtascleral or periorbital routes), as well as those administered systemically with the intent of treating the study and/or fellow eye.

[0053] The study procedures are summarized as follows:

[0054] Best Corrected Visual Acuity: Visual function of the study eye and the fellow eye were assessed using the ETDRS protocol (The Early Treatment Diabetic Retinopathy Study Group) at 4 meters. Visual Acuity examiners were certified to ensure consistent measurement of BCVA. The VA examiners were required to remain masked to treatment assignment.

[0055] Optical Coherence Tomography: Retinal and lesion characteristics were evaluated using OCT on the study eye. At the Screen Visit (Visit 1) images were captured and transmitted for both eyes. All OCT images were captured using the Zeiss Stratus OCT™ with software Version 3 or greater. OCT images were sent to an independent reading center where images were read by masked readers at visits where OCTs were required. All OCTs were electronically archived at the site as part of the source documentation. A subset of OCT images were read. OCT technicians were required to be certified by the reading center to ensure consistency and quality in image acquisition. Adequate efforts were made to ensure that OCT technicians at the site remained masked to treatment assignment.

[0056] Fundus Photography and Fluorescein Angiography (FA): The anatomical state of the retinal vasculature of the study eye was evaluated by funduscopic examination, fundus photography and FA. At the Screen Visit (Visit 1) funduscopic examination, fundus photography and FA were captured and transmitted for both eyes. Fundus and angiographic images were sent to an independent reading center where images were read by masked readers. The reading center confirmed subject eligibility based on angiographic criteria prior to randomization. All FAs and

fundus photographs were archived at the site as part of the source documentation. Photographers were required to be certified by the reading center to ensure consistency and quality in image acquisition. Adequate efforts were made to ensure that all photographers at the site remain masked to treatment assignment.

[0057] Vision-Related Quality of Life: Vision-related QOL was assessed using the National Eye Institute 25-Item Visual Function Questionnaire (NEI VFQ-25) in the interviewer-administered format. NEI VFQ-25 was administered by certified personnel at a contracted call center. At the screening visit, the sites assisted the subject and initiated the first call to the call center to collect all of the subject's contact information and to complete the first NEI VFQ-25 on the phone prior to randomization and IVT injection. For all subsequent visits, the call center called the subject on the phone, prior to IVT injection, to complete the questionnaire.

[0058] Intraocular Pressure: Intraocular pressure (IOP) of the study eye was measured using applanation tonometry or Tonopen. The same method of IOP measurement was used in each subject throughout the study.

[0059]

C. Results Summary (52 Week Data)

[0060] The primary endpoint (prevention of moderate or severe vision loss as defined above) was met for all three VEGFT groups (2Q4, 0.5Q4 and 2Q8) in this study. The results from both studies are summarized in Table 1.

Table 1

	Ranibizumab 0.5 mg monthly (RQ4)	VEGFT 0.5 mg monthly (0.5Q4)	VEGFT 2 mg monthly (2Q4)	VEGFT 2 mg every 8 weeks[a] (2Q8)
Maintenance of vision* (% patients losing <15 letters) at week 52 versus baseline				
Study 1	94.4%	95.9%**	95.1%**	95.1%**
Study 2	94.4%	96.3%**	95.6%**	95.6%**
Mean improvement in vision* (letters) at 52 weeks versus baseline (p-value vs RQ4)***				
Study 1	8.1	6.9 (NS)	10.9 (p<0.01)	7.9 (NS)
Study 2	9.4	9.7 (NS)	7.6 (NS)	8.9 (NS

[a] Following three initial monthly doses
* Visual acuity was measured as the total number of letters read correctly on the Early Treatment Diabetic Retinopathy Study (ETDRS) eye chart.
** Statistically non-inferior based on a non-inferiority margin of 10%, using confidence interval approach (95.1% and 95% for Study 1 and Study 2, respectively)
*** Test for superiority
NS = non-significant

[0061] In Study 1, patients receiving VEGFT 2mg monthly (2Q4) achieved a statistically significant greater mean improvement in visual acuity at week 52 versus baseline (secondary endpoint), compared to ranibizumab 0.5mg monthly (RQ4); patients receiving VEGFT 2mg monthly on average gained 10.9 letters, compared to a mean 8.1 letter gain with ranibizumab 0.5mg dosed every month ($p<0.01$). All other dose groups of VEGFT in Study 1 and all dose groups in Study 2 were not statistically different from ranibizumab in this secondary endpoint.

[0062] A generally favorable safety profile was observed for both VEGFT and ranibizumab. The incidence of ocular treatment emergent adverse events was balanced across all four treatment groups in both studies, with the most frequent events associated with the injection procedure, the underlying disease, and/or the aging process. The most frequent ocular adverse events were conjunctival hemorrhage, macular degeneration, eye pain, retinal hemorrhage, and vitreous floaters. The most frequent serious non-ocular adverse events were typical of those reported in this elderly population who receive intravitreal treatment for wet AMD; the most frequently reported events were falls, pneumonia, myocardial infarction, atrial fibrillation, breast cancer, and acute coronary syndrome. There were no notable differences among the study arms.

Example 5: Phase II Clinical Trial of VEGFT in Subjects with Diabetic Macular Edema (DME)

[0063] In this study, 221 patients with clinically significant DME with central macular involvement were randomized, and 219 patients were treated with balanced distribution over five groups. The control group received macular laser therapy at baseline, and patients were eligible for repeat laser treatments, but no more frequently than at 16 week intervals. The remaining four groups received VEGFT by intravitreal injection as follows: Two groups received 0.5 or 2 mg of VEGFT once every four weeks throughout the 12-month dosing period (0.5Q4 and 2Q4, respectively). Two groups received three initial doses of 2 mg VEGFT once every four weeks (*i.e.*, at baseline, and weeks 4 and 8), followed through week 52 by either once every 8 weeks dosing (2Q8) or as needed dosing with very strict repeat dosing criteria (PRN). Mean gains in visual acuity versus baseline were as shown in Table 2:

Table 2

	n	Mean change in visual acuity at week 24 versus baseline (letters)	Mean change in visual acuity at week 52 versus baseline (letters)
Laser	44	2.5	-1.3
VEGFT 0.5 mg monthly (0.5Q4)	44	8.6**	11.0**
VEGFT 2 mg monthly (2Q4)	44	11.4**	13.1**
VEGFT 2 mg every 8	42	8.5**	9.7**

weeks[a] (2Q8)			
VEGFT 2 mg as needed[a] (PRN)	45	10.3**	12.0**

[a] Following three initial monthly doses
** p < 0.01 versus laser

[0064] In this study, the visual acuity gains achieved with VEGFT administration at week 24 were maintained or numerically improved up to completion of the study at week 52 in all VEGFT study groups, including 2 mg dosed every other month

[0065] As demonstrated in the foregoing Examples, the administration of VEGFT to patients suffering from angiogenic eye disorders (*e.g.*, AMD and DME) at a frequency of once every 8 weeks, following a single initial dose and two secondary doses administered four weeks apart, resulted in significant prevention of moderate or severe vision loss or improvements in visual acuity.

Example 6: A Randomized, Multicenter, Double-Masked Trial in Treatment Naïve Patients with Macular Edema Secondary to CRVO

[0066] In this randomized, double-masked, Phase 3 study, patients received 6 monthly injections of either 2 mg intravitreal VEGFT (114 patients) or sham injections (73 patients). From Week 24 to Week 52, all patients received 2 mg VEGFT as-needed (PRN) according to retreatment criteria. Thus, "sham-treated patients" means patients who received sham injections once every four weeks from Week 0 through Week 20, followed by intravitreal VEGFT as needed from Week 24 through Week 52. "VEGFT-treated patients" means patients who received VEGFT intravitreal injections once every four weeks from Week 0 through Week 20, followed by intravitreal VEGFT as needed from Week 24 through Week 52. The primary endpoint was the proportion of patients who gained ≥15 ETDRS letters from baseline at Week 24. Secondary visual, anatomic, and Quality of Life NEI VFQ-25 outcomes at Weeks 24 and 52 were also evaluated.

[0067] At Week 24, 56.1% of VEGFT-treated patients gained ≥15 ETDRS letters from baseline vs 12.3% of sham-treated patients (P<0.0001). Similarly, at Week 52, 55.3% of VEGFT-treated patients gained ≥15 letters vs 30.1% of sham-treated patients (P<0.01). At Week 52, VEGFT-treated patients gained a mean of 16.2 letters vs 3.8 letters for sham-treated patients (P<0.001). Mean number of injections was 2.7 for VEGFT-treated patients vs 3.9 for sham-treated patients. Mean change in central retinal thickness was -413.0 µm for VEGFT-treated patients vs -381.8 µm for sham-treated patients. The proportion of patients with ocular neovascularization at Week 24 were 0% for VEGFT-treated patients and 6.8% for sham-treated patients, respectively; at Week 52 after receiving VEGFT PRN, proportions were 0% and 6.8% for VEGFT-treated and sham-treated. At Week 24, the mean change from baseline in the VFQ-25 total score was 7.2 vs 0.7 for the

VEGFT-treated and sham-treated groups; at Week 52, the scores were 7.5 vs 5.1 for the VEGFT-treated and sham-treated groups.

[0068] This Example confirms that dosing monthly with 2 mg intravitreal VEGFT injection resulted in a statistically significant improvement in visual acuity at Week 24 that was maintained through Week 52 with PRN dosing compared with sham PRN treatment. VEGFT was generally well tolerated and had a generally favorable safety profile.

Example 7: Dosing Regimens

[0069] Specific, non-limiting examples of dosing regimens within the scope of the present invention are as follows:

[0070] VEGFT 2 mg (0.05 mL) administered by intravitreal injection once every 4 weeks (monthly).

[0071] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 8 weeks, followed by 2 mg (0.05 mL) via intravitreal injection once every 8 weeks.

[0072] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 8 weeks, followed by 2 mg (0.05 mL) via intravitreal injection on a less frequent basis based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0073] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 8 weeks, followed by 2 mg (0.05 mL) via intravitreal injection administered *pro re nata* (PRN) based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0074] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 12 weeks, followed by 2 mg (0.05 mL) via intravitreal injection once every 8 weeks.

[0075] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 12 weeks, followed by 2 mg (0.05 mL) via intravitreal injection on a less frequent basis based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0076] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 12 weeks, followed by 2 mg (0.05 mL) via intravitreal injection administered *pro re nata* (PRN) based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0077] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 16 weeks, followed by 2 mg (0.05 mL) via intravitreal injection once every 8 weeks.

[0078] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 16 weeks, followed by 2 mg (0.05 mL) via intravitreal injection on a less frequent basis based on

visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0079] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 16 weeks, followed by 2 mg (0.05 mL) via intravitreal injection administered *pro re nata* (PRN) based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0080] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 20 weeks, followed by 2 mg (0.05 mL) via intravitreal injection once every 8 weeks.

[0081] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 20 weeks, followed by 2 mg (0.05 mL) via intravitreal injection on a less frequent basis based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0082] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 20 weeks, followed by 2 mg (0.05 mL) via intravitreal injection administered *pro re nata* (PRN) based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0083] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 24 weeks, followed by 2 mg (0.05 mL) via intravitreal injection once every 8 weeks.

[0084] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 24 weeks, followed by 2 mg (0.05 mL) via intravitreal injection on a less frequent basis based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0085] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 24 weeks, followed by 2 mg (0.05 mL) via intravitreal injection administered *pro re nata* (PRN) based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0086] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 28 weeks, followed by 2 mg (0.05 mL) via intravitreal injection once every 8 weeks.

[0087] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 28 weeks, followed by 2 mg (0.05 mL) via intravitreal injection on a less frequent basis based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0088] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 28 weeks, followed by 2 mg (0.05 mL) via intravitreal injection administered *pro re nata* (PRN) based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0089] VEGFT 2 mg (0.05 mL) administered by intravitreal injection as a single initial dose, followed by additional doses administered *pro re nata* (PRN) based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

[0090] Variations on the above-described dosing regimens would be appreciated by persons of ordinary skill in the art and are also within the scope of the present invention. For example, the amount of VEGFT and/or volume of formulation administered to a patient may be varied based on patient characteristics, severity of disease, and other diagnostic assessments by a physician or other qualified medical professional.

[0091] Any of the foregoing administration regimens may be used for the treatment of, *e.g.*, age-related macular degeneration (*e.g.*, wet AMD, exudative AMD, etc.), retinal vein occlusion (RVO), central retinal vein occlusion (CRVO; *e.g.*, macular edema following CRVO), branch retinal vein occlusion (BRVO), diabetic macular edema (DME), choroidal neovascularization (CNV; *e.g.*, myopic CNV), iris neovascularization, neovascular glaucoma, post-surgical fibrosis in glaucoma, proliferative vitreoretinopathy (PVR), optic disc neovascularization, corneal neovascularization, retinal neovascularization, vitreal neovascularization, pannus, pterygium, vascular retinopathy, etc.

SEQUENCES

[0092] SEQ ID NO:1 (DNA sequence having 1377 nucleotides):

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[0093] SEQ ID NO:2 (polypeptide sequence having 458 amino acids):

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[0094] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. A method for treating an angiogenic eye disorder in a patient, said method comprising sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist;

wherein each secondary dose is administered 2 to 4 weeks after the immediately preceding dose; and

wherein each tertiary dose is administered at least 8 weeks after the immediately preceding dose.

2. The method of claim 1, wherein only a single secondary dose is administered to the patient, and wherein the single secondary dose is administered 4 weeks after the initial dose of the VEGF antagonist.

3. The method of claim 1, wherein only two secondary doses are administered to the patient, and wherein each secondary dose is administered 4 weeks after the immediately preceding dose.

4. The method of claim 3, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

5. The method of claim 1, wherein at least 5 tertiary doses of the VEGF antagonist are administered to the patient, and wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered 8 or 12 weeks after the immediately preceding dose.

6. The method of claim 1, wherein the angiogenic eye disorder is selected from the group consisting of: age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization.

7. The method of claim 6, wherein the angiogenic eye disorder is age related macular degeneration.

8. The method of claim 1, wherein the VEGF antagonist is an anti-VEGF antibody or fragment thereof, an anti-VEGF receptor antibody or fragment thereof, or a VEGF receptor-based chimeric molecule.

9. The method of claim 8, wherein the VEGF antagonist is a VEGF receptor-based chimeric molecule.

10. The method of claim 9, wherein the VEGF receptor-based chimeric molecule comprises VEGFR1R2-FcΔC1(a) encoded by the nucleic acid sequence of SEQ ID NO:1.

11. The method of claim 9, wherein the VEGF receptor-based chimeric molecule comprises (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130-231 of SEQ ID NO:2; and (3) a multimerization component comprising amino acids 232-457 of SEQ ID NO:2.

12. The method of claim 1, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

13. The method of claim 12, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

14. The method of claim 13, wherein the intraocular administration is intravitreal administration.

15. The method of claim 11, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

16. The method of claim 15, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

17. The method of claim 16, wherein the intraocular administration is intravitreal administration.

18. The method of claim 17, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

19. The method of claim 18, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

20. The method of claim 18, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

ABSTRACT

The present invention provides methods for treating angiogenic eye disorders by sequentially administering multiple doses of a VEGF antagonist to a patient. The methods of the present invention include the administration of multiple doses of a VEGF antagonist to a patient at a frequency of once every 8 or more weeks. The methods of the present invention are useful for the treatment of angiogenic eye disorders such as age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization.

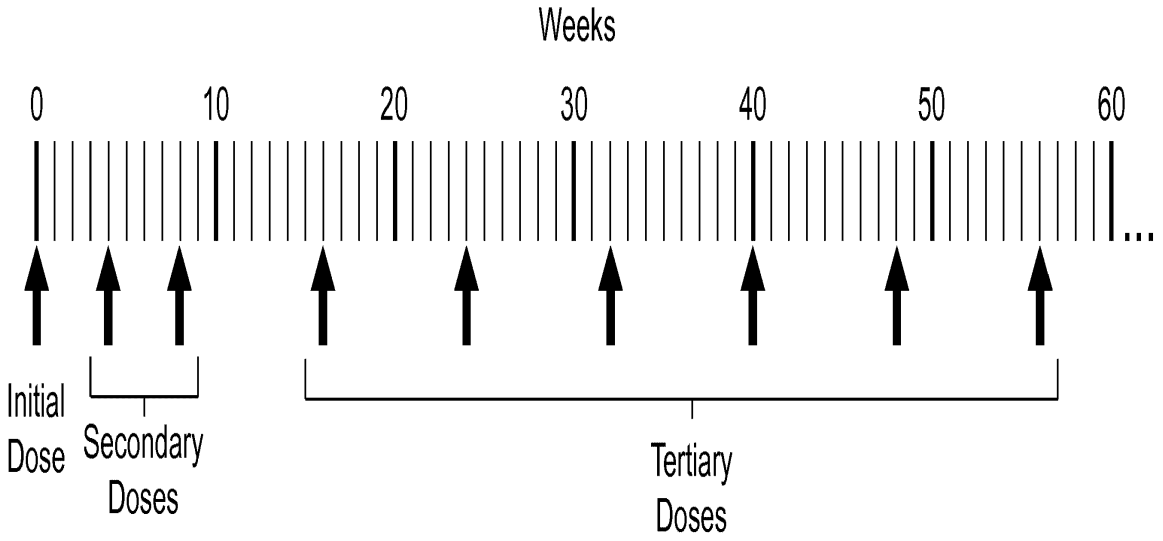

Weeks
0
10
20
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40
50
60
...
Initial Dose
Secondary Doses
Tertiary Doses

Figure 1

SEQUENCE LISTING

<110> George D. Yancopoulos

<120> Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders

<130> 725A1

<140> To be assigned
<141> Filed herewith

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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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Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
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Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
            260   265   270
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
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Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Application of: YANCOPOULOS	Confirmation No.: *To be assigned*
Application No.: *To be assigned*	Art Unit: *To be assigned*
Filed: July 12, 2013	Examiner: *To be assigned*
For: USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	Customer Number: 26693

SEQUENCE LISTING STATEMENT UNDER 37 CFR § 1.821(f)

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

Dear Sir:

In accordance with 37 CFR § 1.821(f), Applicants' undersigned representative hereby states that the sequence listing information recorded in computer readable form, as submitted electronically herewith as a text file, is identical to the paper copy of the sequence listing submitted herewith.

Dated: July 12, 2013

Respectfully submitted,

By */ Frank R. Cottingham /*

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PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	725A1
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

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.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
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Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

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Postal Code	10598	Country i	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

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	96387		
Email Address	bozicevic@bozpat.com	Add Email	Remove Email

Application Information:

Title of the Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS		
Attorney Docket Number	725A1	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	1	Suggested Figure for Publication (if any)	1

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	725A1
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

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).
Either enter 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	96387		

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.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation in part of	PCT/US2012/020855	2012-01-11
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2012/020855	non provisional of	61432245	2011-01-13
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2012/020855	non provisional of	61434836	2011-01-21
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2012/020855	non provisional of	61561957	2011-11-21

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	725A1
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

This section allows for the applicant to claim priority to a foreign application. 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(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) [i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country [i]	Filing Date (YYYY-MM-DD)	Access Code [i] (if applicable)

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

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

PTO/AIA/14 (03-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	725A1
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

Applicant Information:

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

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant 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 [i]	US	Postal Code	10591
Phone Number	914-847-1116	Fax Number	914-847-7705
Email Address	patents@regeneron.com		

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

Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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	725A1
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

Assignee 1

Complete this section only if non-applicant assignee information is desired to be included on the patent application publication in accordance with 37 CFR 1.215(b). Do not include in this section an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest), as the patent application publication will include the name of the applicant(s).

Remove

If the Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

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

Signature:

Remove

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications

Signature	/ Frank R. Cottingham /			Date (YYYY-MM-DD)	2013-07-12
First Name	FRANK	Last Name	COTTINGHAM	Registration Number	50437

Additional Signature may be generated within this form by selecting the Add button. Add

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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 inspections 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.

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 07/29/2013

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If you need help call the Patent Electronic Business Center at (866)
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United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/940,370	07/12/2013	1629	1740	REGN-008CIP (725A1-US)	20	1

CONFIRMATION NO. 1055

96387
Regeneron
Bozicevic, Field & Francis
1900 University Ave
Suite 200
East Palo Alto, CA 94303

FILING RECEIPT

OC000000062895729

Date Mailed: 08/02/2013

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**

Inventor(s)
George D. YANCOPOULOS, Yorktown Heights, NY;

Applicant(s)
REGENERON PHARMACEUTICASS, INC., Tarrytown, NY

Power of Attorney: None

Domestic Priority data as claimed by applicant
This application is a CIP of PCT/US2012/020855 01/11/2012
which claims benefit of 61/432,245 01/13/2011
and claims benefit of 61/434,836 01/21/2011
and claims benefit of 61/561,957 11/21/2011

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 07/29/2013
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/940,370**
Projected Publication Date: 11/07/2013
Non-Publication Request: No

Early Publication Request: No
Title

USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

Preliminary Class

514

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

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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PATENT APPLICATION FEE DETERMINATION RECORD

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Application or Docket Number: 13/940,370

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	20 minus 20 =	*			OR	x 80 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*				x 420 =	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 $310 ($155 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
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1600

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	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))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	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))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/940,370	07/12/2013	George D. YANCOPOULOS	REGN-008CIP (725A1-US)

CONFIRMATION NO. 1055

96387
Regeneron
Bozicevic, Field & Francis
1900 University Ave
Suite 200
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NOTICE

OC00000062895730

Date Mailed: 08/02/2013

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- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
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(Not for submission under 37 CFR 1.99)

Application Number	13940370
Filing Date	2013-07-12
First Named Inventor	YANCOPOULOS, GEORGE D.
Art Unit	
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	2	20050260203		2005-11-24	Wiegand et al.	
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	4	20060172944		2006-08-03	Wiegand et al.	
	5	20070190058		2007-08-16	Shams	

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	1	00/75319	WO		2000-12-14	Regeneron Pharmaceuticals, Inc.		☐

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	1	ANONYMOUS "Lucentis (rangibizymab injection) Intravitreal Injection" pp. 103 (June 2006)	☐
	2	DO et al., "An exploratory study of the safety, tolerability and bioactivity of a single intravitreal injection of vascular endothelial growth factor Trap-Eye in patients with diabetic macular oedema" Br J Opthamol. 93(2):144-1449 (February 2009)	☐
	3	DO et al., "The DA VINCI Study: phase 2 primary results of VEGF Trap-Eye in patients with diabetic macular edema" Opthamology 118(9):1819-1826 (September 2011)	☐
	4	THE EYETECH STUDY GROUP, "Anti-Vascular Endothelial Growth Factor Therapy for Subfoveal Choroidal Neovascularization Secondary to Age-related Macular Degeneration" American Academy of Ophthamology, 110 (5):979-986 (May 2003)	☐
	5	HEIERet al., " rhuFab V2 (anti-VEGF Antibody) for Treatment of Exudative AMD" Symposium 8:Experimental and Emerging Treatments for Choroidal Neovascularization, 10 pp (2002)	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	First Named Inventor	YANCOPOULOS, GEORGE D.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-008CIP

	6	HEIER et al., "RhuFab V2 in Wet AMD - 6 Month Continued Improvement Following Multiple Intravitreal Injections" Invest Ophthalmol Vis Sci, 44:E-Abstract 972 (2003)	☐
	7	KRZYSTOLIK et al., "Prevention of Experimental Choroidal NEovascularization With Intravitreal Anti-Vascular Endothelial Growth Factor Antibody Fragment" Arch Ophthamol., 120:338-346 (Mar. 2002)	☐
	8	NGUYEN et al., "A Phase I Study of Intravitreal Vascular Endothelial Growth Factor Trap-Eye in Patients with Neovascular Age-Related Macular Degeneration" Opthamology, J.B. Lippincott Co., Philadelphia, PA, US, 116(11):2141-2148 (November 1, 2009)	☐
	9	NICHOLS, EARL R., "AAO: Ranibizumab (rhuRab) May Improve Vision in Age-Related Macular Degeneration" Doctor's Guide Global Edition, www.pslgroup.com/dg/23f2aa.htm, pp. 1-2 (November 24, 20013)	☐
	10	PAI et al., "Current concepts in intravitreal drug therapy for diabetic retinopathy" Saudi Journal of Opthamology 24(4):143-149 (June 30, 2010)	☐
	11	STEWART, "THe expanding role of vascular endothelial growth factor inhibitors in opthamology" Mayo Clin Proc. 87 (1):77-88 (January 2012)	☐
	12	THOMAS REUTERS INTEGRITY "VEGF Trap-Eye final phase II results in age-related macular degeneration presented at 2008 Retina Society Meeting" (September 28, 2008)	☐

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	Application Number	13940370
	Filing Date	2013-07-12
	First Named Inventor	YANCOPOULOS, GEORGE D.
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	Examiner Name	
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CERTIFICATION STATEMENT

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

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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	/Karl Bozicevic, Reg. No. 28,807/	Date (YYYY-MM-DD)	2013-10-18
Name/Print	Karl Bozicevic	Registration Number	28807

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(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/75319 A1

(51) International Patent Classification[7]: C12N 15/12, 15/62, 5/10, C07K 14/71, A61K 38/17, A61P 43/00

(21) International Application Number: PCT/US00/14142

(22) International Filing Date: 23 May 2000 (23.05.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/138,133 8 June 1999 (08.06.1999) US

(71) Applicant *(for all designated States except US)*: **REGENERON PHARMACEUTICALS, INC.** [US/US]; 777 Old Saw Mill River Road, Tarrytown, NY 10591-6707 (US).

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(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

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WO 00/75319 A1

(54) Title: MODIFIED CHIMERIC POLYPEPTIDES WITH IMPROVED PHARMACOKINETIC PROPERTIES

(57) Abstract: Modified chimeric polypeptides with improved pharmacokinetics are disclosed. Specifically, modified chimeric Flt1 receptor polypeptides that have been modified in such a way as to improve their pharmacokinetic profile are disclosed. Also disclosed are methods of making and using the modified polypeptides including but not limited to using the modified polypeptides to decrease or inhibit plasma leakage and/or vascular permeability in a mammal.

MODIFIED CHIMERIC POLYPEPTIDES WITH IMPROVED PHARMACOKINETIC PROPERTIES

The application claims priority of U.S. Provisional Application No. 60/138,133, filed on June 8, 1999. Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

INTRODUCTION

The field of this invention is modified polypeptides with improved pharmacokinetics. Specifically, the field of this invention relates to Flt1 receptor polypeptides that have been modified in such a way as to improve their pharmacokinetic profile. The field of this invention also relates to methods of making and using the modified polypeptides including but not limited to using the modified polypeptides to decrease or inhibit plasma leakage and/or vascular permeability in a mammal.

BACKGROUND

The ability of polypeptide ligands to bind to cells and thereby elicit a phenotypic response such as cell growth, survival, cell product secretion, or differentiation is often mediated through transmembrane receptors on the cells. The extracellular domain of such receptors (i.e. that portion of the receptor that is displayed on the surface of the cell) is generally the most distinctive portion of the molecule, as it provides the protein with its ligand binding characteristic. Binding of a ligand

to the extracellular domain generally results in signal transduction which transmits a biological signal to intracellular targets. Often, this signal transduction acts via a catalytic intracellular domain. The particular array of sequence motifs of this catalytic intracellular domain determines its access to potential kinase substrates (Mohammadi, et al.,1990, Mol. Cell. Biol. 11:5068-5078; Fantl, et al., 1992, Cell 69:413-413). Examples of receptors that transduce signals via catalytic intracellular domains include the receptor tyrosine kinases (RTKs) such as the Trk family of receptors which are generally limited to cells of the nervous system, the cytokine family of receptors including the tripartate CNTF receptor complex (Stahl & Yancopoulos, 1994, J. Neurobio. 25:1454-1466) which is also generally limited to the cells of the nervous system, G-protein coupled receptors such as the β_2-adrenergic receptor found on, for instance, cardiac muscle cells, and the multimeric IgE high affinity receptor FcεRI which is localized, for the most part, on mast cells and basophils (Sutton & Gould, 1993, Nature 366:421-428).

All receptors identified so far appear to undergo dimerization, multimerization, or some related conformational change following ligand binding (Schlessinger, J., 1988, Trend Biochem. Sci. 13:443-447; Ullrich & Schlessinger, 1990, Cell 61:203-212; Schlessinger & Ullrich, 1992, Neuron 9:383-391) and molecular interactions between dimerizing intracellular domains lead to activation of catalytic function. In some instances, such as platelet-derived growth factor (PDGF), the ligand is a dimer that binds two receptor molecules (Hart, et al., 1988, Science, 240:1529-1531; Heldin, 1989, J. Biol. Chem. 264:8905-8912) while, for example, in the case of epidermal growth

factor (EGF), the ligand is a monomer (Weber, et al., 1984, J. Biol. Chem. 259:14631-14636). In the case of the FcεRI receptor, the ligand, IgE, exists bound to FcεRI in a monomeric fashion and only becomes activated when antigen binds to the IgE/FcεRI complex and cross-links adjacent IgE molecules (Sutton & Gould, 1993, Nature 366:421-428).

Often, the tissue distribution of a particular receptor within higher organisms provides insight into the biological function of the receptor. The RTKs for some growth and differentiation factors, such as fibroblast growth factor (FGF), are widely expressed and therefore appear to play some general role in tissue growth and maintenance. Members of the Trk RTK family (Glass & Yancopoulos, 1993, Trends in Cell Biol. 3:262-268) of receptors are more generally limited to cells of the nervous system, and the Nerve Growth Factor family consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5), which bind the Trk RTK family receptors, promote the differentiation of diverse groups of neurons in the brain and periphery (Lindsay, R. M, 1993, in Neurotrophic Factors, S.E. Loughlin & J.H. Fallon, eds., pp. 257-284, San Diego, CA, Academic Press). FcεRI is localized to a very limited number of types of cells such as mast cells and basophils. Mast cells derive from bone marrow pluripotent hematopoietic stem cell lineage, but complete their maturation in the tissue following migration from the blood stream (See Janeway & Travers, 1996, in Immunobiology, 2d. Edition, M. Robertson & E. Lawrence, eds., pp. 1:3-1:4, Current Biology Ltd., London, UK, Publisher) and are involved in the allergic response.

Many studies have demonstrated that the extracellular domain of a receptor provides the specific ligand binding characteristic. Furthermore, the cellular environment in which a receptor is expressed may influence the biological response exhibited upon binding of a ligand to the receptor. For example, when a neuronal cell expressing a Trk receptor is exposed to a neurotrophin which binds to that receptor, neuronal survival and differentiation results. When the same receptor is expressed by a fibroblast, exposure to the neurotrophin results in proliferation of the fibroblast (Glass, et al., 1991, Cell 66:405-413).

A class of cell-derived dimeric mitogens with selectivity for vascular endothelial cells has been identified and designated vascular endothelial cell growth factor (VEGF). VEGF has been purified from conditioned growth media of rat glioma cells [Conn et al., (1990), Proc. Natl. Acad. Sci. U.S.A., 87. pp 2628-2632]; and conditioned growth media of bovine pituitary follicle stellate cells [Ferrara and Henzel, (1989), Biochem. Biophys. Res. Comm., 161, pp. 851-858; Gozpadorowicz et al., (1989), Proc. Natl. Acad. Sci. U.S.A., 86, pp. 7311-7315] and conditioned growth medium from human U937 cells [Connolly, D. T. et al. (1989), Science, 246, pp. 1309-1312]. VEGF is a dimer with an apparent molecular mass of about 46 kDa with each subunit having an apparent molecular mass of about 23 kDa. VEGF has some structural similarities to platelet derived growth factor (PDGF), which is a mitogen for connective tissue cells but not mitogenic for vascular endothelial cells from large vessels.

The membrane-bound tyrosine kinase receptor, known as Flt, was shown to be a VEGF receptor [DeVries, C. et al., (1992), Science, 255, pp.989-

991]. The Flt receptor specifically binds VEGF which induces mitogenesis. Another form of the VEGF receptor, designated KDR, is also known to bind VEGF and induce mitogenesis. The partial cDNA sequence and nearly full length protein sequence of KDR is known as well [Terman, B. I. et al., (1991) Oncogene 6, pp. 1677-1683; Terman, B. I. et al., (1992) Biochem. Biophys. Res. Comm. 187, pp. 1579-1586].

Persistent angiogenesis may cause or exacerbate certain diseases such as psoriasis, rheumatoid arthritis, hemangiomas, angiofibromas, diabetic retinopathy and neovascular glaucoma. An inhibitor of VEGF activity would be useful as a treatment for such diseases and other VEGF-induced pathological angiogenesis and vascular permeability conditions, such as tumor vascularization. The present invention relates to a VEGF inhibitor that is based on the VEGF receptor Flt1.

Plasma leakage, a key component of inflammation, occurs in a distinct subset of microvessels. In particular, in most organs plasma leakage occurs specifically in the venules. Unlike arterioles and capillaries, venules become leaky in response to numerous inflammatory mediators including histamine, bradykinin, and serotonin. One characteristic of inflammation is the plasma leakage that results from intercellular gaps that form in the endothelium of venules. Most experimental models of inflammation indicate that these intercellular gaps occur between the endothelial cells of postcapillary and collecting venules (Baluk, P., et al., Am. J. Pathol. 1998 152:1463-76). It has been shown that certain lectins may be used to reveal features of focal sites of plasma leakage, endothelial gaps, and finger-like processes at endothelial cell borders in inflamed venules (Thurston, G., et al., Am. J.

Physiol, 1996, 271: H2547-62). In particular, plant lectins have been used to visualize morphological changes at endothelial cell borders in inflamed venules of, for example, the rat trachea. Lectins, such as conconavalin A and ricin, that bind focally to inflamed venules reveal regions of the subendothelial vessel wall exposed by gaps that correspond to sites of plasma leakage (Thurston, G., et al., Am J Physiol, 1996, 271: H2547-62).

The properties of the microvessels are dynamic. Chronic inflammatory diseases, for example, are associated with microvascular remodeling, including angiogenesis and microvessel enlargement. Microvessels can also remodel by acquiring abnormal phenotypic properties. In a murine model of chronic airway inflammation, airway capillaries acquire properties of venules, including widened vessel diameter, increased immunoreactivity for von Willebrand factor, and increased immunoreactivity for P-selectin. In addition, these remodeled vessels leak in response to inflammatory mediators, whereas vessels in the same position in the airways of normal mice do not.

Certain substances have been shown to decrease or inhibit vascular permeability and/or plasma leakage. For example, mystixins are synthetic polypeptides that have been reported to inhibit plasma leakage without blocking endothelial gap formation (Baluk, P., et al., J. Pharmacol. Exp. Ther., 1998, 284: 693-9). Also, the beta 2-adrenergic receptor agonist formoterol reduces microvascular leakage by inhibiting endothelial gap formation (Baluk, P. and McDonald, D.M., Am. J. Physiol., 1994, 266:L461-8).

The angiopoietins and members of the vascular endothelial growth factor (VEGF) family are the only growth factors thought to be largely specific for vascular endothelial cells. Targeted gene inactivation studies in mice have shown that VEGF is necessary for the early stages of vascular development and that Ang-1 is required for later stages of vascular remodeling.

US Patent No. 6,011,003, issued January 4, 2000, in the name of Metris Therapeutics Limited, discloses an altered, soluble form of FLT polypeptide being capable of binding to VEGF and thereby exerting an inhibitory effect thereon, the polypeptide comprising five or fewer complete immunoglobulin domains.

US Patent No. 5,712,380, issued January 27, 1998 and assigned to Merck & Co., discloses vascular endothelial cell growth factor (VEGF) inhibitors that are naturally occurring or recombinantly engineered soluble forms with or without a C-terminal transmembrane region of the receptor for VEGF.

Also assigned to Merck & Co. is PCT Publication No. WO 98/13071, published April 2, 1998, which discloses gene therapy methodology for inhibition of primary tumor growth and metastasis by gene transfer of a nucleotide sequence encoding a soluble receptor protein which binds to VEGF.

PCT Publication No. WO 97/44453, published November 27, 1997, in the name of Genentech, Inc., discloses novel chimeric VEGF receptor proteins comprising amino acid sequences derived from the vascular

endothelial growth factor (VEGF) receptors Flt1 and KDR, including the murine homologue to the human KDR receptor FLK1, wherein said chimeric VEGF receptor proteins bind to VEGF and antagonize the endothelial cell proliferative and angiogenic activity thereof.

PCT Publication No. WO 97/13787, published April 17, 1997, in the name of Toa Gosei Co., LTD., discloses a low molecular weight VEGF inhibitor usable in the treatment of diseases accompanied by neovascularization such as solid tumors. A polypeptide containing the first immunoglobulin-like domain and the second immunoglobulin-like domain in the extracellular region of a VEGF receptor FLT but not containing the sixth immunoglobulin-like domain and the seventh immunoglobulin-like domain thereof shows a VEGF inhibitory activity.

Sharifi, J. et al., 1998, The Quarterly Jour. of Nucl. Med. 42:242-249, disclose that because monoclonal antibodies (MAbs) are basic, positively charged proteins, and mammalian cells are negatively charged, the electrostatic interactions between the two can create higher levels of background binding resulting in low tumor to normal organ ratios. To overcome this effect, the investigators attempted to improve MAb clearance by using various methods such as secondary agents as well as chemical and charge modifications of the MAb itself.

Jensen-Pippo, et al., 1996, Pharmaceutical Research 13:102-107, disclose that pegylation of a therapeutic protein, recombinant human granulocyte colony stimulating factor (PEG-G-CSF), results in an increase in stability and in retention of *in vivo* bioactivity when administered by the intraduodenal route.

Tsutsumi, *et al.*, 1997, Thromb Haemost. 77:168-73, disclose experiments wherein the *in vivo* thrombopoietic activity of polyethylene glycol-modified interleukin-6 (MPEG-IL-6), in which 54% of the 14 lysine amino groups of IL-6 were coupled with PEG, was compared to that of native IL-6.

Yang, *et al.*, 1995, Cancer 76:687-94, disclose that conjugation of polyethylene glycol to recombinant human interleukin-2 (IL-2) results in a compound, polyethylene glycol-modified IL-2 (PEG-IL-2) that retains the in vitro and in vivo activity of IL-2, but exhibits a markedly prolonged circulating half-life.

R. Duncan and F. Spreafico, Clin. Pharmacokinet. 27: 290-306, 296 (1994) review efforts to improve the plasma half-life of asparaginase by conjugating polyethylene glycol.

PCT International Publication No. WO 99/03996 published January 28, 1999 in the name of Regeneron Pharmaceuticals, Inc. and The Regents of The University of California describes modified human noggin polypeptides having deletions of regions of basic amino acids. The modified human noggin polypeptides are described as retaining biological activity while having reduced affinity for heparin and superior pharmacokinetics in animal sera as compared to the unmodified human noggin.

SUMMARY OF THE INVENTION

The present invention is directed to VEGF antagonists with improved pharmacokinetic properties. A preferred embodiment is an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a VEGF polypeptide comprising (a) a nucleotide sequence encoding a VEGF receptor component operatively linked to (b) a nucleotide sequence encoding a multimerizing component, wherein the VEGF receptor component is the only VEGF receptor component of the fusion polypeptide and wherein the nucleotide sequence of (a) consists essentially of a nucleotide sequence encoding the amino acid sequence of Ig domain 2 of the extracellular domain of a first VEGF receptor and a nucleotide sequence encoding the amino acid sequence of Ig domain 3 of the extracellular domain of a second VEGF receptor.

In a further embodiment, the isolated nucleic acid of the first VEGF receptor is Flt1.

In a further embodiment, the isolated nucleic acid of the second VEGF receptor is Flk1.

In yet another embodiment, the isolated nucleic acid of the second VEGF receptor is Flt4.

In another preferred embodiment, the nucleotide sequence encoding Ig domain 2 of the extracellular domain of the first VEGF receptor is upstream of the nucleotide sequence encoding Ig domain 3 of the extracellular domain of the second VEGF receptor.

In still another preferred embodiment, the nucleotide sequence encoding Ig domain 2 of the extracellular domain of the first VEGF receptor is downstream of the nucleotide sequence encoding Ig domain 3 of the extracellular domain of the second VEGF receptor.

In a preferred embodiment of the invention, the multimerizing component comprises an immunoglobulin domain.

In another embodiment, the immunoglobulin domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

Preferred embodiments include an isolated nucleic acid molecule comprising a nucleotide sequence encoding a modified Flt1 receptor fusion polypeptide, wherein the coding region of the nucleic acid molecule consists of a nucleotide sequence selected from the group consisting of

(a) the nucleotide sequence set forth in Figure 13A-13D;
(b) the nucleotide sequence set forth in Figure 14A-14C;
(c) the nucleotide sequence set forth in Figure 15A-15C;
(d) the nucleotide sequence set forth in Figure 16A-16D;
(e) the nucleotide sequence set forth in Figure 21A-21C;
(f) the nucleotide sequence set forth in Figure 22A-22C;
(g) the nucleotide sequence set forth in Figure 24A-24C; and
(h) a nucleotide sequence which, as a result of the degeneracy of the genetic code, differs from the nucleotide sequence of (a), (b), (c), (d), (e), (f), or (g) and which encodes a fusion polypeptide molecule having

the biological activity of the modified Flt1 receptor fusion polypeptide.

In a further embodiment of the invention, a fusion polypeptide is encoded by the isolated nucleic acid molecules described above.

A preferred embodiment is a composition capable of binding a VEGF molecule to form a nonfunctional complex comprising a multimer of the fusion polypeptide.

Also preferred is a composition wherein the multimer is a dimer.

In yet another embodiment, the composition is in a carrier.

Another embodiment is a vector which comprises the nucleic acid molecules described above, including an expression vector comprising a the nucleic acid molecules described wherein the nucleic acid molecule is operatively linked to an expression control sequence.

Other included embodiments are a host-vector system for the production of a fusion polypeptide which comprises the expression vector, in a suitable host cell; the host-vector system wherein the suitable host cell is a bacterial cell, yeast cell, insect cell, or mammalian cell; the host-vector system wherein the suitable host cell is E. Coli; the host-vector system wherein the suitable host cell is a COS cell; the host-vector system wherein the suitable host cell is a CHO cell.

Another embodiment of the invention is a method of producing a fusion polypeptide which comprises growing cells of the host-vector system under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

Additional embodiments include a fusion polypeptide encoded by the nucleic acid sequence set forth in Figure 10A-10D or Figure 24A-24C, which has been modified by acetylation or pegylation wherein the acetylation is accomplished with at least about a 100 fold molar excess of acetylation reagent or wherein acetylation is accomplished with a molar excess of acetylation reagent ranging from at least about a 10 fold molar excess to about a 100 fold molar excess or wherein the pegylation is 10K or 20K PEG.

A preferred embodiment includes a method of decreasing or inhibiting plasma leakage in a mammal comprising administering to the mammal the fusion polypeptide described above, including embodiments wherein the mammal is a human, the fusion polypeptide is acetylated or the fusion polypeptide is pegylated.

A further embodiments is a fusion polypeptide which specifically binds the VEGF receptor ligand VEGF.

A preferred embodiment of the invention is a method of blocking blood vessel growth in a human comprising administering an effective amount of the fusion polypeptide described above.

Also preferred is a method of inhibiting VEGF receptor ligand activity in a mammal comprising administering to the mammal an effective amount of the fusion polypeptide described above.

Preferred embodiments of these methods are wherein the mammal is a human.

Further embodiments of the methods of the invention include attenuation or prevention of tumor growth in a human; attenuation or prevention of edema in a human, especially wherein the edema is brain edema; attenuation or prevention of ascites formation in a human, especially wherein the ascites is ovarian cancer-associated ascites.

Preferred embodiments of the invention include a fusion polypeptide capable of binding a VEGF polypeptide comprising (a) a VEGF receptor component operatively linked to (b) a multimerizing component, wherein the VEGF receptor component is the only VEGF receptor component in the fusion polypeptide and consists essentially of the amino acid sequence of Ig domain 2 of the extracellular domain of a first VEGF receptor and the amino acid sequence of Ig domain 3 of the extracellular domain of a second VEGF receptor.

In a further embodiment of the fusion polypeptide, the first VEGF receptor is Flt1.

In yet a further embodiment of the fusion polypeptide, the second VEGF receptor is Flk1.

Still another embodiment of the fusion polypeptide is one in which the second VEGF receptor is Flt4.

Preferred embodiments include a fusion polypeptide wherein amino acid sequence of Ig domain 2 of the extracellular domain of the first VEGF receptor is upstream of the amino acid sequence of Ig domain 3 of the extracellular domain of the second VEGF receptor and a fusion polypeptide wherein the amino acid sequence of Ig domain 2 of the extracellular domain of the first VEGF receptor is downstream of the amino acid sequence of Ig domain 3 of the extracellular domain of the second VEGF receptor.

In yet another embodiment, the fusion polypeptide multimerizing component comprises an immunoglobulin domain including an embodiment wherein the immunoglobulin domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

Preferred embodiments include a fusion polypeptide comprising an amino acid sequence of a modified Flt1 receptor, wherein the amino acid sequence selected from the group consisting of (a) the amino acid sequence set forth in Figure 13A-13D; (b) the amino acid sequence set forth in Figure 14A-14C; (c) the amino acid sequence set forth in Figure 15A-15C; (d) the amino acid sequence set forth in Figure 16A-16D; (e) the amino acid sequence set forth in Figure 21A-21C; (f) the amino acid sequence set forth in Figure 22A-22C; and (g) the amino acid sequence set forth in Figure 24A-24C.

Another preferred embodiment is a method of decreasing or inhibiting plasma leakage in a mammal comprising administering to the mammal the fusion polypeptide described above.

An alternative preferred embodiment is a method of inhibiting VEGF receptor ligand activity in a mammal comprising administering to the mammal an effective amount of the fusion polypeptide described above.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. IEF gel analysis of unmodified and acetylated Flt1(1-3)-Fc proteins. Unmodified Flt1(1-3)-Fc protein is unable to enter the gel due to its >9.3 pI, whereas acetylated Flt1(1-3)-Fc is able to enter the gel and equilibrate at pI 5.2.

Figure 2. Binding of unmodified Flt1(1-3)-Fc and acetylated Flt1(1-3)-Fc proteins to Matrigel® coated plates. Unmodified Flt1(1-3)-Fc proteins binds extensive to extracellular matrix components in Matrigel®, whereas acetylated Flt1(1-3)-Fc does not bind.

Figure 3. Binding of unmodified Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc, and pegylated Flt1(1-3)-Fc in a Biacore-based assay. Acetylated (columns 13-16), pegylated (columns 17-20), and heparin-treated Flt1(1-3)-Fc (columns 21-24) are each able to completely compete with the Biacore chip-bound Flt1(1-3)-Fc for VEGF binding as compared to control (columns 1-4) and irrelevant protein (columns 5-8). Unmodified Flt1(1-3)-Fc (columns 5-6) appears to only partially compete with Biacore chip-bound Flt1(1-3)-Fc for VEGF binding.

However, washing the bound samples with 0.5M NaCl (columns 7-8) results in a binding profile similar to the modified forms of Flt1(1-3)-Fc, indicating that the unmodified protein is exhibiting non-specific binding to the chip that can be eliminated by the salt wash.

Figure 4. Binding of unmodified Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc, and pegylated Flt1(1-3)-Fc to VEGF in an ELISA-based assay. Both pegylated and acetylated Flt1(1-3)-Fc proteins bind to VEGF with affinities approaching that of unmodified Flt1(1-3)-Fc.

Figure 5. Pharmacokinetic profiles of unmodified Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc, and pegylated Flt1(1-3)-Fc. Balb/c mice (23-28g) were injected subcutaneously with 4mg/kg of unmodified, acetylated, or pegylated Flt1(1-3)-Fc. The mice were tail bled at 1, 2, 4, 6, 24 hours, 2 days, and 3 days after injection of protein and the sera were assayed in a standard ELISA-based assay designed to detect Flt1(1-3)-Fc protein. The T_{max} for all of the Flt1(1-3)-Fc proteins was between the 6 hour and 24 hour time points. The C_{max} for the different proteins was as follows: Unmodified: 0.06 μg/ml - 0.15 μg/ml; acetylated: 1.5 μg/ml - 4.0 μg/ml; and pegylated: approximately 5 μg/ml.

Figure 6A-6B. IEF gel analysis of unmodified and step-acetylated Flt1(1-3)-Fc proteins. Unmodified Flt1(1-3)-Fc protein is unable to enter the gel due to its >9.3 pI, whereas most of the step-acetylated Flt1(1-3)-Fc samples (30-100 fold excess samples) were able to migrate into the gel and equilibrate at pIs ranging between 4.55 - 8.43, depending on the degree of acetylation.

Figure 7. Binding of unmodified Flt1(1-3)-Fc and step-acetylated Flt1(1-3)-Fc proteins to Matrigel® coated plates. As with the irrelevant control protein, rTie2-Fc, step-acetylated Flt1(1-3)-Fc (20 and 30 fold excess samples) does not exhibit any binding to the Matrigel coated plate, whereas the non-acetylated Flt1(1-3)-Fc protein exhibits significant binding. The 10 fold excess sample shows reduced binding, but the degree of acetylation is not enough to completely block binding to extracellular matrix components.

Figure 8. Binding of unmodified Flt1(1-3)-Fc and step-acetylated Flt1(1-3)-Fc in a Biacore-based assay. At a sub-stoichiometric ratio (0.5 μg/ml of either unmodified Flt1(1-3) or step-acetylated Flt1(1-3)-Fc vs. 0.2 μg/ml VEGF), there is not enough Flt1(1-3)-Fc (either unmodified or step-acetylated) in the solution to completely bind the VEGF. At 1.0 μg/ml, which approximates a 1:1 stoichiometric ratio, the both unmodified and step-acetylated Flt1(1-3)-Fc are better able to compete for VEGF binding, but there is still insufficient Flt1(1-3)-Fc protein (either unmodified or step-acetylated) to completely saturate the available VEGF. However, at 5.0 μg/ml, which is several times greater than a 1:1 stoichiometric ratio, both the Flt1(1-3)-Fc and the step-acetylated Flt1(1-3)-Fc proteins are able to saturate the VEGF, regardless of the degree of acetylation.

Figure 9. Pharmacokinetic profiles of unmodified Flt1(1-3)-Fc and step-acetylated Flt1(1-3)-Fc. Balb/c mice (23-28g) were injected subcutaneously with 4mg/kg of unmodified or 10, 20, 40, 60 and 100 fold excess samples of step-acetylated Flt1(1-3)-Fc (3 mice for

unmodified, 10, 20 and 40 fold excess samples and 2 mice for 60 and 100 fold excess samples). The mice were tail bled at 1, 2, 4, 6, 24 hours, 2 days and 3 days after injection. The sera were assayed in an ELISA-based assay designed to detect Flt1(1-3)-Fc. The T_{max} for all of the Flt1(1-3)-Fc proteins tested was at the 6 hour time point but the C_{max} was as follows: Unmodified Flt1(1-3)-Fc: 0.06μg/ml; 10 fold excess sample: - 0.7μg/ml, 20 fold excess sample - 2μg/ml, 40 fold excess sample - 4μg/ml, 60 fold excess sample - 2μg/ml, 100 fold excess sample - 1μg/ml.

Figure 10A-10D. Nucleic acid and deduced amino acid sequence of Flt1(1-3)-Fc.

Figure 11. Schematic diagram of the structure of Flt1.

Figure 12A and 12B. Hydrophilicity analysis of the amino acid sequences of Ig domain 2 and Ig domain 3 of Flt1.

Figure 13A-13D. Nucleic acid and deduced amino acid sequence of Mut1: Flt1(1-$3_{\Delta B}$)-Fc.

Figure 14A-14 C. Nucleic acid and deduced amino acid sequence of Mut2: Flt1(2-$3_{\Delta B}$)-Fc.

Figure 15A-15C. Nucleic acid and deduced amino acid sequence of Mut3: Flt1(2-3)-Fc.

Figure 16A-16D. Nucleic acid and deduced amino acid sequence of Mut4: Flt1(1-3$_{R->N}$)-Fc.

Figure 17. Binding of unmodified Flt1(1-3)-Fc, basic region deletion mutant Flt1(1-3)-Fc, and Flt1(1-3)$_{R->N}$ mutant proteins in a Biacore-based assay. At the sub-stoichiometric ratio (0.25 μg/ml Flt1(1-3)-Fc of unmodified, acetylated or genetically modified samples vs. 01. μg/ml VEGF), there is insufficient Flt1(1-3)-Fc protein to block binding of VEGF to the Flt1(1-3)-Fc immobilized on the Biacore chip. At 0.5 μg/ml of unmodified, acetylated or genetically modified Flt1(1-3)-Fc proteins, the stoichiometric ratio approximates 1:1 and there is an increased ability to block VEGF binding to the Biacore chip. At 1.0 μg/ml of unmodified, acetylated or genetically modified Flt1(1-3)-Fc proteins, which is approximately a 10:1 stoichiometric ratio, the Flt1(1-3)-Fc proteins are able to block binding of VEGF to the Biacore chip, but they are not equivalent. Unmodified, acetylated, and Mut1: Flt1(1-3$_{\Delta B}$)-Fc are essentially equal in their ability to block VEGF binding, whereas Mut4: Flt1(1-3$_{R->N}$)-Fc is somewhat less efficient at blocking binding

Figure 18. Binding of unmodified Flt1(1-3)-Fc, Mut1: Flt1(1-3$_{\Delta B}$)-Fc, Mut2: Flt1(2-3$_{\Delta B}$)-Fc, and Flt1(2-3) mutant proteins to Matrigel® coated plates. Unmodified Flt1(1-3)-Fc protein binds avidly to these wells, the Mut3: Flt1(2-3)-Fc protein binds somewhat more weakly, the Mut1: Flt1(1-3$_{\Delta B}$)-Fc protein binds more weakly still, and the Mut2: Flt1(2-3$_{\Delta B}$)-Fc protein shows the best profile, binding more weakly than any of the other mutant proteins. The Mut4: Flt1(1-3$_{R->N}$)-Fc

glycosylation mutant protein shows only marginal benefit on the Matrigel assay.

Figure 19. Binding of unmodified Flt1(1-3)-Fc, Mut1: Flt1(1-$3_{\Delta B}$)-Fc, Mut2: Flt1(2-$3_{\Delta B}$)-Fc, and Flt1(2-3) mutant proteins in an ELISA-based assay. At the concentrations tested, unmodified Flt1(1-3)-Fc, Mut1: Flt1(1-$3_{\Delta B}$)-Fc, Mut2: Flt1(2-$3_{\Delta B}$)-Fc, and Flt1(2-3) mutant proteins bind VEGF similarly.

Figure 20. Pharmacokinetic profiles of unmodified Flt1(1-3)-Fc, Mut1: Flt1(1-$3_{\Delta B}$)-Fc, Mut2: Flt1(2-$3_{\Delta B}$)-Fc, and Flt1(2-3) mutant proteins. the Cmax for these reagents was as follows: Unmodified Flt1(1-3)-Fc - 0.15μg/ml; 40 fold molar excess acetylated Flt1(1-3)-Fc - 1.5μg/ml; and Mut1: Flt1(1-$3_{\Delta B}$)-Fc - 0.7μg/ml.

Figure 21A-21C. Nucleotide and deduced amino acid sequence of the modified Flt1 receptor termed Flt1D2.Flk1D3.FcΔC1(a).

Figure 22A-22C. Nucleotide and deduced amino acid sequence of the modified Flt1 receptor termed Flt1D2.VEGFR3D3.FcΔC1(a).

Figure 23. Extracellular Matrix (ECM) Assay. The results of this assay demonstrate that the Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a) proteins are considerably less sticky to the ECM as compared to the Flt1(1-3)-Fc protein.

Figure 24A-24C. Nucleotide and deduced amino acid sequence of the modified Flt1 receptor termed VEGFR1R2-FcΔC1(a).

Figure 25A-25C. Phosphorylation assay. At a 1.5 molar excess of either Flt1(1-3)-Fc , Flt1(1-3)-Fc (A40) or transient Flt1D2Flk1D3.FcΔC1(a) there is complete blockage of receptor stimulation by these three modified Flt1 receptors as compared to control media challenge. In contrast, transient Flt1D2VEGFR3D3.FcΔC1(a) does not show significant blockage at this molar excess, as compared with VEGF positive control challenge. Similar results are seen in Figure 25B, where the modified Flt receptors are in a 3-fold molar excess to VEGF165 ligand. In Figure 25C, where the modified Flt1 receptors are in a 6-fold molar excess to VEGF165 ligand, transient Flt1D2VEGFR3D3.FcΔC1(a) can now be shown to be partially blocking VEGF165-induced stimulation of cell-surface receptors.

Figure 26A-26B. Phosphorylation assay. Detection by Western blot of tyrosine phosphorylated VEGFR2(Flk1) by VEGF165 ligand stimulation shows that cell-surface receptors are not phosphorylated by challenge samples which have VEGF165 preincubated with 1 and 2 fold molar excess (Figure 26A) or 3 and 4 fold molar excess (Figure 26B) of either transient Flt1D2Flk1D3.FcΔC1(a), stable Flt1D2Flk1D3.FcΔC1(a), or transient VEGFR1R2-FcΔC1(a). At all modified Flt1 receptor concentrations tested there is complete binding of VEGF165 ligand

during the preincubation, resulting in no detectable stimulation of cell-surface receptors by unbound VEGF165 as compared to control media challenge.

Figure 27. MG/R2 Cell proliferation assay. The following modified Flt receptors Flt1(1-3)-Fc, Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a), plus an irrelevant receptor termed Tie2-Fc as a negative control, were titrated from 40nM to 20pM and incubated on the cells for 1hr at 37°C. Human recombinant VEGF165 in defined media was then added to all the wells at a concentration of 1.56nM. The negative control receptor Tie2-Fc does not block VEGF165-induced cell proliferation at any concentration whereas Flt1D2.Flk1D3.FcΔC1(a) blocks 1.56nM VEGF165 with a half maximal dose of 0.8nM. Flt1(1-3)-Fc and Flt1D2.VEGFR3D3.FcΔC1(a) are less effective in blocking VEGF165 in this assay with a half maximal dose of ~ 2nM. VEGF165 alone gives a reading of 1.2 absorbance units and the background is 0.38 absorbance units.

Figure 28. Biacore analysis of Binding Stoichiometry. Binding stoichiometry was calculated as a molar ratio of bound VEGF165 to the immobilized Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a), using the conversion factor of 1000 RU equivalent to 1 ng/ml. The results indicated binding stoichiometry of one VEGF165 dimeric molecule per one Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) molecule.

Figure 29 and Figure 30. Size Exclusion Chromatography Stoichiometry. Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) at a concentration of 1nM (estimated to be 1000 times higher than the KD of the Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a)/VEGF165 interaction) were mixed with varied concentrations of VEGF165. After incubation, concentrations of the free Flt1D2Flk1D3.FcΔC1(a) in solution were measured. The data shows that the addition of 1 nM VEGF165 into the Flt1D2Flk1D3.FcΔC1(a) solution completely blocks Flt1D2Flk1D3.FcΔC1(a) binding to the VEGF165 surface. This result suggested the binding stoichiometry of one VEGF165 molecule per one Flt1D2Flk1D3.FcΔC1(a) molecule.

Figure 31. Size Exclusion Chromatography (SEC) under native conditions. Peak #1 represents the Flt1D2Flk1D3.FcΔC1(a)/ VEGF165 complex and peak #2 represents unbound VEGF165. Fractions eluted between 1.1 and 1.2 ml were combined and guanidinium hydrochloride (GuHCl)was added to a final concentration 4.5M to dissociate the complex.

Figure 32. Size Exclusion Chromatography (SEC) under dissociative conditions. To separate the components of the receptor-ligand complex and to determine their molar ratio, 50μl of dissociated complex was loaded onto a Superose 12 PC 3.2/30 equilibrated in 6M GuHCl and eluted. Peak #1 represents Flt1D2Flk1D3.FcΔC1(a) and peak #2 represents VEGF165.

Figure 33, Figure 34 and Figure 35. Size Exclusion Chromatography (SEC) with On-Line Light Scattering. Size exclusion chromatography column with a MiniDawn on-line light scattering detector (Wyatt Technology, Santa Barbara, California) and refractive index (RI) detectors (Shimadzu, Kyoto, Japan) was used to determine the molecular weight (MW) of the receptor-ligand complex. As shown in Figure 33, the elution profile shows two peaks. Peak #1 represents the receptor-ligand complex and peak #2 represents the unbound VEGF165. MW was calculated from LS and RI signals. The same procedure was used to determine MW of the individual components of the receptor-ligand complex. The results of these determinations are as follows: MW of the Flt1D2Flk1D3.FcΔC1(a)/VEGF165 complex at the peak position is 157 300 (Figure 33), the MW of VEGF165 at the peak position is 44 390 (Figure 34) and the MW of R1R2 at the peak is 113 300 (Figure 35).

Figure 36. Peptide mapping and glycosylation analysis. The disulfide structures and glycosylation sites in Flt1D2.Flk1D3.FcΔC1(a) were determined by a peptide mapping method. There are a total of ten cysteines in Flt1D2.Flk1D3.FcΔC1(a); six of them belong to the Fc region. Cys27 is disulfide bonded to Cys76. Cys121 is disulfide bonded to Cys 182. The first two cysteines in the Fc region (Cys211 and Cys214) form an intermolecular disulfide bond with the same two cysteines in another Fc chain. However, it can not be determined

whether disulfide bonding is occurring between same cysteines (Cys211 to Cys211, for example) or between Cys211 and Cys214. Cys216 is disulfide bonded to Cys306. Cys 352 is disulfide bonded to Cys410.

There are five possible N-linked glycosylation sites in Flt1D2.Flk1D3.FcΔC1(a) and are found to be glycosylated to varying degrees. Complete glycosylation is observed at Asn33, Asn193, and Asn282. Partial glycosylation is observed on Asn65 and Asn120. Sites of glycosylation are highlighted by underline in the Figure.

Figure 37. Pharmacokinetics of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a). Balb/c mice were injected subcutaneously with 4mg/kg of Flt1(1-3)-Fc (A40), CHO transiently expressed Flt1D2.Flk1D3.FcΔC1(a), CHO stably expressed Flt1D2.Flk1D3.FcΔC1(a), and CHO transiently expressed VEGFR1R2-FcΔC1(a). The mice were tail bled at 1, 2, 4, 6, 24 hrs, 2 days, 3 days and 6 days after injection. The sera were assayed in an ELISA designed to detect Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a). The Tmax for Flt1(1-3)-Fc (A40) was at 6 hrs while the Tmax for the transient and stable Flt1D2.Flk1D3.FcΔC1(a) and the transient VEGFR1R2-FcΔC1(a) was 24hrs. The Cmax for Flt1(1-3)-Fc (A40) was 8μg/ml, For both transients (Flt1D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a)) the Cmax was 18μg/ml and the Cmax for the stable VEGFR1R2-FcΔC1(a) was 30μg/ml.

Figure 38. Pharmacokinetics of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a). Balb/c mice were injected subcutaneously with 4mg/kg of Flt1(1-3)-Fc (A40), CHO transiently expressed Flt1D2.Flk1D3.FcΔC1(a) and CHO transiently expressed Flt1D2.VEGFR3D3.FcΔC1(a). The mice were tail bled at 1, 2, 5, 6, 7, 8, 12, 15 and 20 days after injection. The sera were assayed in an ELISA designed to detect Flt1(1-3)-Fc, Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a). Flt1(1-3)-Fc (A40) could no longer be detected in the serum after day 5 whereas Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a) were detectable for 15 days or more.

Figure 39. The Ability of Flt1D2.Flk1D3.FcΔC1(a) to Inhibit HT-1080 Fibrosarcoma Tumor Growth In Vivo. Every other day or 2 times per week treatment of SCID mice with Flt1D2.Flk1D3.FcΔC1(a) at 25mg/Kg significantly decreases the growth of subcutaneous HT-1080 fibrosarcoma tumors.

Figure 40. The Ability of Flt1D2.Flk1D3.FcΔC1(a) to Inhibit C6 Glioma Tumor Growth In Vivo. Every other day or 2 times a week treatment of SCID mice with Flt1D2.Flk1D3.FcΔC1(a) significantly decreases the growth of subcutaneous C6 glioma tumors at doses as low as 2.5mg/Kg.

Figure 41. VEGF-Induced Uterine Hyperpermeability. PMSG injected subcutaneously (5 IU) to induce ovulation in prepubertal female rats

results in a surge of estradiol after 2 days which in turn causes an induction of VEGF in the uterus. This induction results in hyperpermeability of the uterus and an increase in uterine wet. Subcutaneous injection of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a) at 25mg/kg at 1hr after PMSG injection results in about a 50% inhibition of the increase in uterine wet weight.

Figure 42A-42B. Assessment of Corpus Luteum Angiogenesis Using Progesterone as a Readout. PMSG was injected subcutaneously (5 IU) to induce ovulation in prepubertal female rats, resulting in a fully functioning corpus luteum containing a dense network of blood vessels that secretes progesterone into the blood stream to prepare the uterus for implantation. The induction of angiogenesis in the corpus luteum requires VEGF. Resting levels of progesterone are about 5ng/ml and can be induced to 25-40ng/ml after PMSG. Subcutaneous injection of Flt1(1-3)-Fc (A40) or Flt1D2.Flk1D3.FcΔC1(a) at 25mg/kg or 5mg/kg at 1hr. after PMSG injection resulted in a complete inhibition of the progesterone induction on day 4.

DETAILED DESCRIPTION OF THE INVENTION

It has been a long standing problem in the art to produce a receptor based VEGF antagonist that has a pharmacokinetic profile that is appropriate for consideration of the antagonist as a therapeutic candidate. Applicants describe herein, for the first time, a chimeric

polypeptide molecule, capable of antagonizing VEGF activity, that exhibits improved pharmacokinetic properties as compared to other known receptor-based VEGF antagonists. The chimeric polypeptide molecules described herein thus provide for the first time appropriate molecules for use in therapies in which antagonism of VEGF is a desired result.

The present invention provides for novel chimeric polypeptide molecules formed by fusing a modified extracellular ligand binding domain of the Flt1 receptor to the Fc region of IgG.

The extracellular ligand binding domain is defined as the portion of a receptor that, in its native conformation in the cell membrane, is oriented extracellularly where it can contact with its cognate ligand. The extracellular ligand binding domain does not include the hydrophobic amino acids associated with the receptor's transmembrane domain or any amino acids associated with the receptor's intracellular domain. Generally, the intracellular or cytoplasmic domain of a receptor is usually composed of positively charged or polar amino acids (i.e. lysine, arginine, histidine, glutamic acid, aspartic acid). The preceding 15-30, predominantly hydrophobic or apolar amino acids (i.e. leucine, valine, isoleucine, and phenylalanine) comprise the transmembrane domain. The extracellular domain comprises the amino acids that precede the hydrophobic transmembrane stretch of amino acids. Usually the transmembrane domain is flanked by positively

charged or polar amino acids such as lysine or arginine. von Heijne has published detailed rules that are commonly referred to by skilled artisans when determining which amino acids of a given receptor belong to the extracellular, transmembrane, or intracellular domains (See von Heijne, 1995, BioEssays 17:25-30). Alternatively, websites on the Internet, such as http://ulrec3.unil.ch/software/TMPRED_form.html. have become available to provide protein chemists with information about making predictions about protein domains.

The present invention provides for the construction of nucleic acid molecules encoding chimeric polypeptide molecules that are inserted into a vector that is able to express the chimeric polypeptide molecules when introduced into an appropriate host cell. Appropriate host cells include, but are not limited to, bacterial cells, yeast cells, insect cells, 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 chimeric polypeptide molecules under control of transcriptional/translational control signals. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombinations (genetic recombination) (See Sambrook, et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory; Current Protocols in Molecular Biology, Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY).

Expression of nucleic acid molecules encoding the chimeric polypeptide molecules may be regulated by a second nucleic acid sequence so that the chimeric polypeptide molecule is expressed in a host transformed with the recombinant DNA molecule. For example, expression of the chimeric polypeptide molecules described herein 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, the long terminal repeat as described in Squinto et al., (1991, Cell 65:1-20); the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the CMV promoter, the M-MuLV 5' terminal repeat the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:144-1445), the regulatory sequences of the metallothionine gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25, see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADH (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and

have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58); alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al, 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Thus, according to the invention, expression vectors capable of being replicated in a bacterial or eukaryotic host comprising chimeric polypeptide molecule-encoding nucleic acid as described herein, are used to transfect the host and thereby direct expression of such nucleic acids to produce the chimeric polypeptide molecules, which may then be recovered in a biologically active form. As used herein, a biologically active form includes a form capable of binding to VEGF.

Expression vectors containing the chimeric nucleic acid molecules described herein can be identified by three general approaches: (a) DNA-DNA hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign gene inserted in an expression vector can be detected by DNA-DNA hybridization using probes comprising sequences that are homologous to the inserted chimeric polypeptide molecule sequences. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. For example, if the chimeric polypeptide molecule DNA sequence is inserted within the marker gene sequence of the vector, recombinants containing the insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying

the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the chimeric polypeptide molecules.

Cells of the present invention may transiently or, preferably, constitutively and permanently express the chimeric polypeptide molecules.

The chimeric polypeptide molecules may be purified by any technique which allows for the subsequent formation of a stable, biologically active chimeric polypeptide molecule. 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, Builder, et al., 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.

In one embodiment of the invention, the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component. In another embodiment of the invention, the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component. Further embodiments of the invention may be prepared in which the order of the

first, second and third fusion polypeptide components are rearranged. For example, if the nucleotide sequence encoding the first component is designated 1, the nucleotide sequence encoding the second component is designated 2, and the nucleotide sequence of the third component is designated 3, then the order of the components in the isolated nucleic acid of the invention as read from 5' to 3' may be any of the following six combinations: 1,2,3; 1,3,2; 2,1,3; 2,3,1; 3,1,2; or 3,2,1.

The present invention also has diagnostic and therapeutic utilities. In particular embodiments of the invention, methods of detecting aberrancies in the function or expression of the chimeric polypeptide molecules described herein may be used in the diagnosis of disorders. In other embodiments, manipulation of the chimeric polypeptide molecules or agonists or antagonists which bind the chimeric polypeptide molecules may be used in the treatment of diseases. In further embodiments, the chimeric polypeptide molecule is utilized as an agent to block the binding of a binding agent to its target.

By way of example, but not limitation, the method of the invention may be useful in treating clinical conditions that are characterized by 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.

An amino acid sequence analysis of Flt1(1-3)-Fc revealed the presence of an unusually high number (46) of the basic amino acid residue lysine. An IEF analysis of Flt1(1-3)-Fc showed that this protein has pI greater than 9.3, confirming the prediction that the protein is very basic. It was hypothesized that the basic nature of Flt1(1-3)-Fc protein was causing it to bind to extracellular matrix components and that this interaction might be the cause of the extremely short detectable circulating serum half-life exhibited by Flt1(1-3)-Fc when injected into mice. In order to test this hypothesis, Flt1(1-3)-Fc protein was acetylated at the lysine residues to reduce the basic charge. Acetylated Flt1(1-3)-Fc was then tested in the assays described *infra*.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1: Expression of Flt1(1-3)-Fc protein in CHO K1 cells.

Using standard molecular biology techniques (see e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor

Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY), the gene encoding Flt1(1-3)-Fc was inserted into the expression vector pEE14.1 (Lonza Biologics, plc) at a multiple cloning site downstream of the CMV promoter. CHO K1 cells were transfected with the pEE14.1/Flt1(1-3)-Fc DNA construct using lipofectamine (Gaithersburg, MD). The transfected CHO K1 cells were grown in glutamine-free DMEM (JRH, Kansas City, MO) containing 25μM methionine sulfoximine (MSX) from Sigma Inc., St. Louis, MO, and high recombinant protein expressors were obtained by screening the CHO K1 cell supernatants from over 100 hand-picked colony isolates using a standard immunoassay which captures and detects human Fc. The selected hand-picked clone was amplified in the presence of 100 μM MSX followed by a second round of screening of the amplified clones. The highest producing clone had a specific productivity of recombinant Flt1(1-3)-Fc protein of 55 pg/cell/day.

The selected clone was expanded in 225cm^2 T-flasks (Corning, Acton, MA) and then into 8.5L roller bottles (Corning, Acton, MA) using the cell culture media described *supra*. Cells were removed from the roller bottles by standard trypsinization and put into 3.5L of suspension medium. The suspension medium is comprised of glutamine-free ISCHO medium (Irvine Scientific, Santa Ana, CA) containing 5% fetal bovine serum (FBS from Hyclone Labs, Logan, UT), 100μM MSX and GS supplement (JRH Scientific, Kansas City, MO) in a 5L Celligen bioreactor (New Brunswick Scientific, New Brunswick, NJ) at a density

of 0.3 x 10^6 cells/mL. After the cells reached a density of 3.6 x 10^6/mL and were adapted to suspension they were transferred to a 60L bioreactor (ABEC, Allentown, PA) at a density of 0.5 x 10^6 cells/mL in 20L of ISCHO medium with 5% fetal bovine serum. After two days an additional 20L of ISCHO + 5% fetal bovine serum was added to the bioreactor. The cells were allowed to grow for an additional two days reaching a final density of 3.1 x 10^6 cells/mL, and a final Flt1(1-3)-Fc concentration at harvest was 95 mg/L. At harvest the cells were removed by tangential flow filtration using 0.45μm Prostak Filters (Millipore, Inc., Bedford, MA).

Example 2: Purification of Flt1(1-3)-Fc protein obtained from CHO K1 cells

Flt1(1-3)-Fc protein was initially purified by affinity chromatography. A Protein A column was used to bind, with high specificity, the Fc portion of the molecule. This affinity-purified protein was then concentrated and passed over a SEC column. The protein was then eluted into the formulation buffer. The following describes these procedures in detail.

Materials and Methods

All chemicals were obtained from J.T. Baker, Phillipsburg, NJ with the exception of PBS, which was obtained as a 10X concentrate from Life

Technologies, Gaithersburg, MD. Protein A Fast Flow and Superdex 200 preparation grade resins were obtained from Pharmacia, Piscataway, NJ. Equipment and membranes for protein concentration were obtained from Millipore, Bedford, MA.

Approximately 40L of 0.45μm-filtered CHO conditioned media containing Flt1(1-3)-Fc protein was applied to a 290mL Protein A Fast Flow column (10cm diameter) that had been equilibrated with PBS. The column was washed with PBS containing 350mM NaCl and 0.02% CHAPS and the bound protein was eluted with 20mM Citric Acid containing 10mM Na_2HPO_4. The single peak in the elution was collected and its pH was raised to neutrality with 1M NaOH. The eluate fractions was concentrated to approximately 9 mg/mL using 10K regenerated cellulose membranes by both tangential flow filtration and by stirred cell concentration. To remove aggregates and other contaminants, the concentrated protein was applied to a column packed with Superdex 200 preparation grade resin (10cm x 55cm) and run in PBS containing 5 % glycerol. The main peak fractions were pooled, sterile filtered, aliquoted and stored at -80°C.

Example 3: Acetylation of Flt1(1-3)-Fc protein.

Two milligrams of Flt1(1-3)-Fc protein were acetylated as described in the instruction manual provided with the sulfo-NHS-acetate modification kit (Pierce Chemical Co., Rockford, IL, Cat.#26777).

Example 4: Characterization of acetylated Flt1(1-3)-Fc protein.

(a.) IEF analysis: Flt1(1-3)-Fc and acetylated Flt1(1-3)-Fc were analyzed by standard IEF analysis. As shown in Figure 1, Flt1(1-3)-Fc protein is not able to migrate into the gel and therefore must have a pI greater than 9.3, the highest pI in the standard. However, acetylated Flt1(1-3)-Fc is able to migrate into the gel and equilibrate at a pI of approximately 5.2. This result demonstrates that acetylation reduces the net positive charge of the protein and therefore its pI considerably.

(b.) Binding to extracellular matrix components

To test for binding to extracellular matrix components, Flt1(1-3)-Fc and acetylated Flt1(1-3)-Fc where tested in an assay designed to mimic the interaction with extracellular matrix components. In this assay, 96-well tissue culture plates are coated with Matrigel (Biocoat MATRIGEL® matrix thin layer 96 well plate, Catalog #40607, Becton Dickinson Labware, Bedford, MA). The plates are incubated with varying concentrations of either Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc, or rTie2-Fc (an irrelevant control) protein are added to the wells. The plates are incubated for 1-2 hours at either room temperature or 37°C degrees and then detection of bound proteins is accomplished by adding a secondary alkaline phosphatase-conjugated anti-human Fc antibody to

the wells. Finally, alkaline phosphatase substrate is added to the wells and optical density is measured. Figure 2 shows the results of this assay. Like the irrelevant control protein rTie2-Fc, acetylated Flt1(1-3)-Fc does not exhibit any binding to the Matrigel coated plate, whereas the non-acetylated Flt1(1-3)-Fc protein exhibits significant binding. This result indicates that acetylation of basic amino acid residues is an effective way to interfere with the charge interactions that exist between positively charged proteins and the negatively charged extracellular matrix components they are exposed to *in vivo*.

Example 5: Pegylation of Flt1(1-3)-Fc protein.

Although pegylation (polyethylene glycol - PEG) of proteins has been shown to increase their *in vivo* potency by enhancing stability and bioavailability while minimizing immunogenicity (see references cited *supra*), it is counter-intuitive that pegylating molecules that are too large to be filtered by the kidney glomeruli would improve their pharmacokinetic properties. Without being bound by theory, Applicants postulated that pegylation of the Flt1(1-3)-Fc molecules could improve the pharmacokinetic properties, possibly not by altering the positive charge or by decreasing the pI of Flt1(1-3)-Fc, but rather by physically shielding the positive charges from interacting with the extracellular matrix. Applicants decided to attempt to improve the pharmacokinetic properties of Flt1(1-3)-Fc molecules by attaching strands of 20K PEGs as described *infra*.

Materials and Methods

Purified Flt1(1-3)-Fc derived from CHO cells (see *supra*) was used in the following pegylation experiments. Functionalized PEGs were obtained from Shearwater Polymers, Huntsville, AL; Bicine from Sigma, St Louis, MO; Superose 6 column from Pharmacia, Piscataway, NJ; PBS as a 10X concentrate from Life Technologies, Gaithersburg, MD; Glycerol from J.T. Baker, Phillipsburg, NJ; and Bis-Tris precast gels from Novex, CA.

20K PEG strands functionalized with amine-specific terminal moieties were used in small-scale reaction studies that were set-up to evaluate different reaction conditions in which the PEG:protein stoichiometry was varied. Based on these reactions and the analyses of samples on standard SDS-PAGE, Flt1(1-3)-Fc at a concentration of 1.5 mg/mL was reacted at pH 8.1 with 20K SPA-PEG (PEG succinimidyl propionate) molecules at a PEG-to-Flt1(1-3)-Fc monomer molar ratio of 1:6. The reaction was allowed to proceed at 8°C overnight. For initial purification, the reaction products were applied to a 10mm x 30cm Superose 6 column equilibrated with PBS containing 5% Glycerol. The column appeared to separate pegylated Flt1(1-3)-Fc molecules based on the extent of pegylation. Fractions corresponding to what appeared to be primarily mono-pegylated and di-pegylated dimeric Flt1(1-3)-Fc, as judged by banding patterns on reducing and non-reducing SDS-PAGE gels

were pooled. The protein concentration was determined by measuring absorbance at 280 nm. The pegylated Flt1(1-3)-Fc protein was sterile filtered, aliquoted and stored at -40°C.

Example 6: Binding of unmodified, acetylated, and pegylated Flt1(1-3)-Fc in a Biacore-based assay.

Unmodified, acetylated, and pegylated Flt1(1-3)-Fc proteins were tested in a Biacore-based assay to evaluate their ability to bind to the Flt1 ligand, VEGF. In this assay, unmodified Flt1(1-3)-Fc protein was immobilized on the surface of a Biacore chip (see Biacore Instruction Manual, Pharmacia, Inc., Piscataway, NJ, for standard procedures) and a sample containing 0.2 μg/ml VEGF and either unmodified Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc or pegylated Flt1(1-3)-Fc (each at 25 μg/ml) was passed over the Flt1(1-3)-Fc-coated chip. To minimize the effects of non-specific binding, the bound samples were washed with a 0.5M NaCl wash. In one sample, unmodified Flt1(1-3)-Fc was mixed with heparin. Heparin is a negatively charged molecule and the Flt1(1-3)-Fc protein is a positively charged molecule, so when the two molecules are mixed together, they should interact through their respective charges. This essentially neutralizes Flt1(1-3)-Fc's inherent positive charge making the molecule behave as if it has been chemically or genetically modified so as to reduce its charge and its tendency to bind via charge interactions. As shown in Figure 3, acetylated (columns 13-16), pegylated (columns 17-20), and heparin-treated Flt1(1-3)-Fc

(columns 21-24) are each able to completely compete with the Biacore chip-bound Flt1(1-3)-Fc for VEGF binding as compared to control (columns 1-4) and irrelevant protein (columns 5-8). Unmodified Flt1(1-3)-Fc (columns 5-6) appeared to only partially compete with Biacore chip-bound Flt1(1-3)-Fc for VEGF binding. However, washing the bound samples with 0.5M NaCl (columns 7-8) resulted in a binding profile similar to the modified forms of Flt1(1-3)-Fc, indicating that the unmodified protein was exhibiting non-specific binding to the chip that could be eliminated by the salt wash.

Example 7: Binding of unmodified, acetylated, and pegylated Flt1(1-3)-Fc in an ELISA-based assay.

Unmodified, acetylated, and pegylated Flt1(1-3)-Fc proteins were tested in a standard ELISA-based assay to evaluate their ability to bind the Flt1 receptor ligand VEGF. As shown in Figure 4, both pegylated and acetylated Flt1(1-3)-Fc proteins are capable of binding to VEGF, demonstrating that modifying the protein either by pegylation or acetylation does not destroy its ability to bind its ligand.

Example 8: Pharmacokinetic analysis of unmodified Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc, and pegylated Flt1(1-3)-Fc.

In vivo experiments were designed to assess the pharmacokinetic profiles of unmodified Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc, and

pegylated Flt1(1-3)-Fc protein. Balb/c mice (23-28g; 3 mice/group) were injected subcutaneously with 4mg/kg of unmodified, acetylated, or pegylated Flt1(1-3)-Fc. The mice were tail bled at 1, 2, 4, 6, 24 hours, 2 days, and 3 days after injection of protein. The sera were assayed in a standard ELISA-based assay designed to detect Flt1(1-3)-Fc protein. Briefly, the assay involves coating an ELISA plate with VEGF, binding the unmodified, acetylated, or pegylated Flt1(1-3)-Fc-containing sera, and reporting with an anti-Fc antibody linked to alkaline phosphatase. As shown in Figure 5, the Tmax for all of the Flt1(1-3)-Fc proteins was between the 6 hour and 24 hour time points. The Cmax for the different proteins was as follows: Unmodified: 0.06 μ/ml - 0.15 μg/ml; acetylated: 1.5 μg/ml - 4.0 μg/ml; and pegylated: approximately 5 μg/ml.

Example 9: Step-acetylation of Flt1(1-3)-Fc

To determine what minimal amount of acetylation is necessary to eliminate binding to extracellular matrix components, an experiment was designed that acetylated the Flt1(1-3)-Fc protein in a step-wise fashion by using increasing amounts of molar excess of acetylation reagent in the acetylation reaction mixture. The range of molar excess was as follows: 0, 10, 20, 30, 40, 50, 60, 70, 80 , 90, and 100 moles of acetylation reagent per 1 mole of Flt1(1-3)-Fc monomer. The reactions were performed as detailed in the instruction manual provided with the

sulfo-NHS-Acetate modification kit (Pierce Chemical Co., Rockford, IL, Cat.# 26777).

Example 10: Characterization of step-acetylated Flt1(1-3)-Fc.

(a.) IEF analysis Unmodified Flt1(1-3)-Fc and step-acetylated Flt1(1-3)-Fc proteins were analyzed by standard IEF analysis. As shown in Figure 6A-6B, unmodified Flt1(1-3)-Fc protein was not able to migrate into the gel due to its extremely high pI (greater than 9.3). However, most of the step-acetylated Flt1(1-3)-Fc samples (30-100 fold molar excess samples) were able to migrate into the gel and equilibrate at pIs ranging between 4.55 - 8.43, depending on the degree of acetylation of the protein. This result demonstrates that acetylation can change the positive charge of the protein in a dose-dependent manner and that reduction of the pI can be controlled by controlling the degree of acetylation.

(b.) Binding of step-acetylated Flt1(1-3)-Fc to extracellular matrix components

To test for binding to extracellular matrix components, Flt1(1-3)-Fc and step-acetylated Flt1(1-3)-Fc where tested in the above-described assay designed to mimic the interaction with extracellular matrix components. Varying concentrations of either unmodified Flt1(1-3)-Fc,

step-acetylated Flt1(1-3)-Fc (10, 20, and 30 fold molar excess samples), or rTie2-Fc (an irrelevant control) protein were added to the wells. The plates were incubated for 1-2 hours at room temperature or 37°C and then detection of bound proteins was accomplished by adding a secondary alkaline phosphatase-conjugated anti-human Fc antibody to the wells. Alkaline phosphatase substrate was subsequently added to the wells and optical density measured. Figure 7 shows the results of this assay. Like the irrelevant control protein rTie2-Fc, step-acetylated Flt1(1-3)-Fc (20 and 30 fold molar excess samples) did not exhibit any significant binding to the Matrigel coated plate, whereas the non-acetylated Flt1(1-3)-Fc protein exhibited significant binding. The binding is saturable, indicating that the Flt1(1-3)-Fc protein may be binding to specific sites, rather than a more general charge-mediated interaction that might not be saturable. The 10 fold molar excess sample showed reduced binding, but the degree of acetylation was not enough to completely block binding to extracellular matrix components. The 20 fold molar excess and higher samples displayed no detectable binding, despite the fact that by IEF analysis (Figure 6A and 6B) the lower molar excess samples still had a large net positive charge. This result demonstrates that it is not necessary to completely acetylate all available basic amino acids in order to eliminate binding to extracellular matrix components.

(c.) Binding of step-acetylated Flt1(1-3)-Fc in a Biacore-based assay.

Unmodified and step-acetylated Flt1(1-3)-Fc proteins where tested in a Biacore-based assay to evaluate their ability to bind to the Flt1 ligand, VEGF. In this assay, unmodified Flt1(1-3)-Fc protein (0.5, 1.0, or 5.0 μg/ml) was immobilized on the surface of a Biacore chip (see Biacore Instruction Manual, Pharmacia, Inc., Piscataway, NJ, for standard procedures) and a solution containing 0.2 μg/ml VEGF and either unmodified Flt1(1-3)-Fc (at either 0.5, 1.0, or 5.0 μg/ml) or 10 different step-acetylated Flt1(1-3)-Fc samples (at 0.5, 1.0, or 5.0 μg/ml each) were passed over the Flt1(1-3)-Fc-coated chip. As shown in Figure 8, at a sub-stoichiometric ratio (0.5 μg/ml of either unmodified Flt1(1-3) or step-acetylated Flt1(1-3)-Fc vs. 0.2 μg/ml VEGF), there is not enough Flt1(1-3)-Fc (either unmodified or step-acetylated) in the solution to completely bind the VEGF. At 1.0 μg/ml, which approximates a 1:1 stoichiometric ratio, both unmodified and step-acetylated Flt1(1-3)-Fc are better able to compete for VEGF binding, but there is still insufficient Flt1(1-3)-Fc protein (either unmodified or step-acetylated) to completely bind the available VEGF. However, at 5.0 μg/ml, which is several times greater than a 1:1 stoichiometric ratio, both the Flt1(1-3)-Fc and the step-acetylated Flt1(1-3)-Fc proteins are able to bind the VEGF, regardless of the degree of acetylation. This clearly demonstrates that acetylation does not alter Flt1(1-3)-Fc's ability to bind VEGF.

(d.) Pharmacokinetic analysis of step-acetylated Flt1(1-3)-Fc

In vivo experiments were designed to assess the pharmacokinetic profiles of unmodified Flt1(1-3)-Fc and step-acetylated Flt1(1-3)-Fc protein. Balb/c mice (23-28g) were injected subcutaneously with 4mg/kg of unmodified or 10, 20, 40, 60 and 100 fold molar excess samples of step-acetylated Flt1(1-3)-Fc (3 mice for unmodified, 10, 20 and 40 fold molar excess samples and 2 mice for 60 and 100 fold molar excess samples). The mice were tail bled at 1, 2, 4, 6, 24 hours, 2 days and 3 days after injection. The sera were assayed in an ELISA-based assay designed to detect Flt1(1-3)-Fc (described *supra*). Figure 9 details the results of this study. The Tmax for all of the Flt1(1-3)-Fc proteins tested was at the 6 hour time point but the Cmax was as follows: Unmodified Flt1(1-3)-Fc: 0.06μg/ml; 10 fold molar excess sample: - 0.7μg/ml, 20 fold molar excess sample - 2μg/ml, 40 fold molar excess sample - 4μg/ml, 60 fold molar excess sample - 2μg/ml, 100 fold molar excess sample - 1μg/ml. This results demonstrates that acetylation or pegylation of Flt1(1-3)-Fc significantly improves its pharmacokinetic profile.

Example 11: Construction of Flt1(1-3)-Fc basic region deletion mutant designated Mut1: Flt1(1-3$_{\Delta B}$)-Fc.

Based on the observation that acetylated Flt1(1-3)-Fc, which has a pI

below 6, has much better pharmacokinetics than the highly positive unmodified Flt1(1-3)-Fc (pI > 9.3), it was asked whether the difference in pharmacokinetics could be attributed to the net charge of the protein, which made it stick to negatively charged extracellular matrix components, or whether there were perhaps specific locations on the surface of the Flt1(1-3)-Fc protein that constituted specific binding sites for extracellular matrix components. For example, many proteins are known to have heparin binding sites, often consisting of a cluster of basic residues. Sometimes these residues are found in a cluster on the primary sequence of the protein; some of the literature has identified "consensus sequences" for such heparin binding sites (see for example Hileman, et al., 1998, Bioessays 20(2):156-67). In other cases, the known crystal structure of a protein reveals a cluster of positively charged residues on the surface of a protein, but the residues come from different regions of the primary sequence and are only brought together when the protein folds into its tertiary structure. Thus it is difficult to deduce whether an isolated amino acid residue forms part of a cluster of basic residues on the surface of the protein. However, if there is a cluster of positively charged amino acid residues in the primary sequence, it is not unreasonable to surmise that the residues are spatially close to one another and might therefore be part of an extracellular matrix component binding site. Flt1 receptor has been studied extensively and various domains have been described (see for example Tanaka et al., 1997, Jpn. J. Cancer Res 88:867-876). Referring to the nucleic acid and amino acid sequence set forth in Figure 10A-10D

of this application, one can identify the signal sequence for secretion which is located at the beginning of the sequence and extends to the glycine coded for by nucleotides 76-78. The mature protein begins with Ser-Lys-Leu-Lys, starting at nucleotide 79 of the nucleic acid sequence. Flt1 Ig domain 1 extends from nucleotide 79 to 393, ending with the amino acids Ser-Asp-Thr. Flt1 Ig domain 2 extends from nucleotide 394 to 687 (encoding Gly-Arg-Pro to Asn-Thr-Ile), and Flt1 Ig domain 3 extends from nucleotides 688 to 996 (encoding Ile-Asp-Val to Asp-Lys-Ala). There is a bridging amino acid sequence, Gly-Pro-Gly, encoded by nucleotides 997-1005, followed by the nucleotide sequence encoding human Fc (nucleotides 1006-1701 or amino acids Glu-Pro-Lys to Pro-Gly-Lys-stop).

A more detailed analysis of the Flt1 amino acid sequence reveals that there is a cluster, namely, amino acid residues 272-281 (KNKRASVRR) of Figure 10A-10D, in which 6 out of 10 amino acid residues are basic. This sequence is located in Flt1 Ig domain 3 of the receptor (see Figure 11), which is not itself essential for binding of VEGF ligand, but which confers a higher affinity binding to ligand. An alignment of the sequence of Ig domain 3 with that of Ig domain 2 reveals that in this region, there is very poor alignment between the two Ig domains, and that there are about 10 additional amino acids in Ig domain 3. An analysis of the hydrophilicity profiles (MacVector computer software) of these two domains clearly indicates the presence of a hydrophilic region in the protein (Figure 12A-12B). These observations raised the

possibility that the actual three dimensional conformation of Flt1 Ig domain 3 allowed for some type of protrusion that is not in Flt1 Ig domain 2. To test this hypothesis, the 10 additional amino acids were deleted and the resulting protein was tested to see whether the deletion would affect the pharmacokinetics favorably without seriously compromising the affinity of the receptor for VEGF. This DNA construct, which was constructed using standard molecular biology techniques (see e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY) in the mammalian expression vector pMT21 (Genetics Institute, Inc., Cambridge, MA), is referred to as Mut1: Flt1(1-3$_{\Delta B}$)-Fc. The Mut1: Flt1(1-3$_{\Delta B}$)-Fc construct was derived from Flt1(1-3)-Fc by deletion of nucleotides 814-843 (set forth in Figure 10A-10D), which deletes the highly basic 10-amino acid residue sequence Lys-Asn-Lys-Arg-Ala-Ser-Val-Arg-Arg-Arg from Flt1 Ig domain 3.

The final DNA construct was sequence-verified using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). The sequence of Mut1: Flt1(1-3$_{\Delta B}$)-Fc is set forth in Figure 13A-13D.

Example 12: Construction of Flt1(1-3)-Fc basic region deletion mutant designated Mut2: Flt1(2-3 $_{\Delta B}$)-Fc.

A second deletion mutant construct, designated Mut2: Flt1(2-3$_{\Delta B}$)-Fc, was derived from the Mut1: Flt1(1-3$_{\Delta B}$)-Fc construct by deletion of Flt1 Ig domain 1 encoded by nucleotides 79-393 (see Figure 10A-10D); for convenience, nucleotides 73-78 (TCA GGT) were changed to TCC GGA. This introduced a restriction site (BspE1) without altering the associated amino acid sequence, Ser-Gly. This DNA construct, which was constructed using standard molecular biology techniques (see e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY) in the mammalian expression vector pMT21 (Genetics Institute, Inc., Cambridge, MA), was also sequence-verified using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). The sequence of Mut2: Flt1(2-3$_{\Delta B}$)-Fc is set forth in Figure 14A-14C.

Example 13: Construction of Flt1(1-3)-Fc deletion mutant designated Mut3: Flt1(2-3)-Fc.

A third deletion mutate construct, designated Mut3: Flt1(2-3)-Fc, was constructed the same way as the Mut2: Flt1(2-3$_{\Delta B}$)-Fc construct, except that Flt1 Ig domain 3 was left intact (the basic region amino

acids were not deleted). The construct was constructed using standard molecular biology techniques and the final construct was sequence-verified as described *supra*. The sequence of Mut3: Flt1(2-3)-Fc is set forth in Figure 15A-15C.

Example 14: Construction of Flt(1-3)-Fc basic region N-glycosylation mutant designated Mut4: Flt1(1-3 $_{R->N}$)-Fc.

A final construct was made in which a N-glycosylation site was introduced into the middle of the basic region of Flt1 Ig domain 3. This construct was designated Mut4: Flt1(1-3$_{R->N}$)-Fc and was made by changing nucleotides 824-825 from GA to AC, consequently changing the coded Arg residue (AGA) into an Asn residue (AAC) (see Figure 10A-10D). The resulting amino acid sequence is therefore changed from Arg-Ala-Ser to Asn-Ala-Ser, which matches the canonical signal (Asn-Xxx-Ser/Thr) for the addition of a N-glycosylation site at the Asn residue. The sequence of Mut4: Flt1(1-3$_{R->N}$)-Fc is set forth in Figure 16A-16D.

Example 15: Characterization of acetylated Flt1(1-3)-Fc, Mut1: Flt1(1-3$_{\Delta B}$)-Fc, and Mut4: Flt1(1-3$_{R->N}$)-Fc mutants.

(a.) Binding to extracellular matrix components

To determine whether the three modified proteins were more or less

likely to have improved pharmacokinetic properties, Matrigel coated 96-well dishes (as described *supra*) were incubated with varying concentrations of the mutant proteins and detected with anti-human Fc/alkaline-phosphatase conjugated antibodies. As shown in Figure 18, this experiment showed that while the unmodified Flt1(1-3)-Fc protein could bind avidly to these wells, the Mut3: Flt1(2-3)-Fc protein bound somewhat more weakly, the Mut1: Flt1(1-$3_{\Delta B}$)-Fc protein bound more weakly still, and the Mut2: Flt1(2-$3_{\Delta B}$)-Fc protein showed the best profile, binding more weakly than any of the other mutant proteins. The Mut4: Flt1(1-$3_{R->N}$)-Fc glycosylation mutant protein showed only marginal benefit on the Matrigel assay. These results confirm the hypothesis that a linear sequence of positive amino acids can be deleted from the primary sequence resulting in a decrease in charge interaction with extracellular matrix components.

(b.) Binding of Mut1: Flt1(1-$3_{\Delta B}$)-Fc and Mut4: Flt1(1-$3_{R->N}$)-Fc in a Biacore-based assay.

Unmodified and acetylated Flt1(1-3)-Fc and genetically modified Mut1: Flt1(1-$3_{\Delta B}$)-Fc and Mut4: Flt1(1-$3_{R->N}$)-Fc proteins where tested in a Biacore-based assay to evaluate their ability to bind to the Flt1 ligand, VEGF. In this assay, unmodified Flt1(1-3)-Fc protein (0.25, 0.5, or 1.0 μg/ml) was immobilized on the surface of a Biacore chip (see Biacore Instruction Manual, Pharmacia, Inc., Piscataway, NJ, for standard procedures) and a solution containing 0.1 μg/ml VEGF and either

purified or COS cell supernatant containing unmodified Flt1(1-3)-Fc (at approximately (0.25, 0.5, or 1.0 μg/ml), purified acetylated Flt1(1-3)-Fc (at (0.25, 0.5, or 1.0 μg/ml), COS cell supernatant containing Mut1: Flt1(1-$3_{\Delta B}$)-Fc (at approximately (0.25, 0.5, or 1.0 μg/ml), or COS cell supernatant containing Mut4: Flt1(1-$3_{R->N}$)-Fc (at approximately (0.25, 0.5, or 1.0 μg/ml) were passed over the Flt1(1-3)-Fc-coated chip. As shown in Figure 17, at the sub-stoichiometric ratio (0.25 μg/ml Flt1(1-3)-Fc of unmodified, acetylated or genetically modified samples vs. 01. μg/ml VEGF), there is insufficient Flt1(1-3)-Fc protein to block binding of VEGF to the Flt1(1-3)-Fc immobilized on the Biacore chip. At 0.5 μg/ml of unmodified, acetylated or genetically modified Flt1(1-3)-Fc proteins, the stoichiometric ratio approximates 1:1 and there is an increased ability to block VEGF binding to the Biacore chip. At 1.0 μg/ml of unmodified, acetylated or genetically modified Flt1(1-3)-Fc proteins, which is approximately a 10:1 stoichiometric ratio, the Flt1(1-3)-Fc proteins are able to block binding of VEGF to the Biacore chip, but they are not equivalent. Unmodified, acetylated, and Mut1: Flt1(1-$3_{\Delta B}$)-Fc are essentially equal in their ability to block VEGF binding, whereas Mut4: Flt1(1-$3_{R->N}$)-Fc is somewhat less efficient at blocking binding. These results confirm the hypothesis that it is possible to reduce the non-specific binding of a positively charged molecule by genetically removing a linear sequence of predominantly negatively charged amino acids.

(c.) Binding of Mut1: Flt1(1-3$_{\Delta B}$)-Fc, Mut2: Flt1(2-3$_{\Delta B}$)-Fc, Mut3: Flt1(2-3)-Fc, and in an ELISA-based assay.

To determine whether the three mutant proteins could bind the Flt1 ligand VEGF, binding experiments were done in which 96-well plates coated with VEGF were incubated with varying concentrations of the respective mutant protein, and after washing, the amount bound was detected by incubating with an alkaline phosphatase conjugated anti-human Fc antibody and quantitated colorimetrically by the addition of an appropriate alkaline phosphatase substrate. As shown in Figure 19, this experiment showed that all the mutant proteins could bind VEGF similarly, at the concentrations tested.

Example 16: Pharmacokinetic analysis of acetylated Flt1(1-3)-Fc, Mut1: Flt1(1-3$_{\Delta B}$)-Fc, and unmodified Flt1(1-3)-Fc.

In vivo experiments were designed to assess the pharmacokinetic profiles of unmodified Flt1(1-3)-Fc, Mut1: Flt1(1-3$_{\Delta B}$)-Fc, and 40 fold molar excess acetylated Flt1(1-3)-Fc protein. Balb/c mice (25-30g) were injected subcutaneously with 4mg/kg of unmodified Flt1(1-3)-Fc, 40 fold molar excess acetylated Flt1(1-3)-Fc, and Mut1: Flt1(1-3$_{\Delta B}$)-Fc proteins (4 mice each). These mice were tail bled at 1, 2, 4, 6, 24 hours, 2 days, 3 days, and 5 days after injection. The sera were assayed in an ELISA designed to detect Flt1(1-3)-Fc protein which involves coating an ELISA plate with VEGF, binding the Flt1(1-3)-Fc and

reporting with an anti-Fc antibody linked to alkaline phosphatase. As shown in Figure 20, the Cmax for these reagents was as follows: Unmodified Flt1(1-3)-Fc - 0.15μg/ml; 40 fold molar excess acetylated Flt1(1-3)-Fc - 1.5μg/ml; and Mut1: Flt1(1-$3_{\Delta B}$)-Fc - 0.7μg/ml.

Example 17: Modified Flt1 receptor vector construction

The rationale for constructing modified versions of the Flt1 receptor (also known as VEGFR1) was based on the observation that the protein sequence of Flt1 was highly basic, and was therefore likely to stick to extracellular matrix (ECM). The highly basic nature of Flt1 probably explains why unmodified Flt1(1-3)-Fc (described *supra*) has poor pharmacokinetics that make it difficult to use as a therapeutic agent. As described *supra*, the chemically modified form of 40 fold molar excess acetylated Flt1(1-3)-Fc, hereinafter termed A40, exhibited a greatly improved pharmacokinetic (PK) profile over the non-acetylated Flt1(1-3)-Fc. Therefore, attempts were made to engineer DNA molecules that could be used to recombinantly express modified forms of a Flt1 receptor molecule that would possess the improved PK profile exhibited by A40 and still maintain the ability to bind tightly to VEGF.

It is known in the literature that the first Ig domain of Flt1 (which has a net charge of +5 at neutral pH) is not essential for tight binding to VEGF, so this domain was deleted. The third Ig domain (having a net charge of +11) is not essential for binding, but confers higher affinity

for VEGF than the second Ig domain, so instead of deleting it entirely, it was replaced with the equivalent domains of the Flt1 receptor relatives Flk1 (also known as VEGFR2) and Flt4 (also known as VEGFR3). These chimeric molecules (denoted R1R2 (Flt1.D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a) and R1R3 (Flt1D2.VEGFR3D3-FcΔC1(a) and VEGFR1R3-FcΔC1(a) respectively, wherein R1 and Flt1D2 = Ig domain 2 of Flt1 (VEGFR1); R2 and Flk1D3 = Ig domain 3 of Flk1 (VEGFR2); and R3 and VEGFR3D3 = Ig domain 3 of Flt4 (VEGFR3)) were much less sticky to ECM, as judged by an *in vitro* ECM binding assay as described *infra* , had greatly improved PK as described *infra*. In addition, these molecules were able to bind VEGF tightly as described *infra* and block phosphorylation of the native Flk1 receptor expressed in endothelial cells as described *infra*.

(a) Construction of the expression plasmid pFlt1D2.Flk1D3.FcΔC1(a)

Expression plasmids pMT21.Flt1(1-3).Fc (6519bp) and pMT21.Flk-1(1-3).Fc (5230bp) are plasmids that encode ampicillin resistance and Fc-tagged versions of Ig domains 1-3 of human Flt1 and human Flk1, respectively. These plasmids were used to construct a DNA fragment consisting of a fusion of Ig domain 2 of Flt1 with Ig domain 3 of Flk1, using PCR amplification of the respective Ig domains followed by further rounds of PCR to achieve fusion of the two domains into a

single fragment. For Ig domain 2 of Flt1, the 5' and 3' amplification primers were as follows:

5': bsp/flt1D2 (5'-GACTAGCAGTCCGGAGGTAGACCTTTCGTAGAGATG-3')

3': Flt1D2-Flk1D3.as (5'-CGGACTCAGAACCACATCTATGATTGTATTGGT-3')

The 5' amplification primer encodes a BspE1 restriction enzyme site upstream of Ig domain 2 of Flt1, defined by the amino acid sequence GRPFVEM (corresponding to amino acids 27-33 of Figure 21A-21C). The 3' primer encodes the reverse complement of the 3' end of Flt1 Ig domain 2 fused directly to the 5' beginning of Flk1 Ig domain 3, with the fusion point defined as TIID of Flt1 (corresponding to amino acids 123-126 of Figure 21A-21C) and continuing into VVLS (corresponding to amino acids 127-130 of Figure 21A-21C) of Flk1.

For Ig domain 3 of Flk1, the 5' and 3' amplification primers were as follows:

5': Flt1D2-Flk1D3.s (5'-ACAATCATAGATGTGGTTCTGAGTCCGTCTCATG G-3')

3': Flk1D3/apa/srf.as (5'-GATAATGCCCGGGCCCTTTTCATGGACCCTGAC AAATG-3')

The 5' amplification primer encodes the end of Flt1 Ig domain 2 fused directly to the beginning of Flk1 Ig domain 3, as described above. The 3' amplification primer encodes the end of Flk1 Ig domain 3, defined by the amino acids VRVHEK (corresponding to amino acids 223-228 of Figure 21A-21C), followed by a bridging sequence that includes a recognition sequence for the restriction enzyme Srf1, and encodes the amino acids GPG. The bridging sequence corresponds to amino acids 229-231 of Figure 21A-21C.

After a round of PCR amplification to produce the individual domains, the products were combined in a tube and subjected to a further round of PCR with the primers bsp/flt1D2 and Flk1D3/apa/srf.as (described *supra*) to produce the fusion product. This PCR product was subsequently digested with the restriction enzymes BspEI and SmaI and the resulting 614bp fragment was subcloned into the BspEI to SrfI restriction sites of the vector pMT21/ΔB2.Fc, to create the plasmid pMT21/Flt1D2.Flk1D3.Fc. The nucleotide sequence of the Flt1D2-Flk1D3 gene fusion insert was verified by standard sequence analysis. This plasmid was then digested with the restriction enzymes EcoRI and SrfI and the resulting 702bp fragment was transferred into the EcoRI to SrfI restriction sites of the plasmid pFlt1(1-3)B2-FcΔC1(a) to produce the plasmid pFlt1D2.Flk1D3.FcΔC1(a). The complete DNA and deduced amino acid sequences of the Flt1D2.Flk1D3.FcΔC1(a) chimeric molecule is set forth in Figure 21A-21C.

(b) Construction of the expression plasmid pFlt1D2VEGFR3D3FcΔC1(a)

The expression plasmid pMT21.Flt1(1-3).Fc (6519bp) encodes ampicillin resistance and an Fc-tagged version of Ig domains 1-3 of human Flt1 receptor. This plasmid was used to produce a DNA fragment containing Ig domain 2 of Flt1 by PCR. RNA from the cell line HEL921.7 was used to produce Ig domain 3 of Flk1, using standard RT-PCR methodology. A further round of PCR amplification was used to achieve fusion of the two Ig domains into a single fused fragment. For Ig domain 2 of Flt1, the 5' and 3' amplification primers were as follows:

5': bsp/flt1D2 (5'-GACTAGCAGTCCGGAGGTAGACCTTTCGTAGAGATG-3')

3': Flt1D2.VEGFR3D3.as(TTCCTGGGCAACAGCTGGATATCTATGATTGTA TTGGT)

The 5' amplification primer encodes a BspE1 restriction site upstream of Ig domain 2 of Flt1, defined by the amino acid sequence GRPFVEM (corresponding to amino acids 27-33 of Figure 22A-22C). The 3' amplification primer encodes the reverse complement of the end of Flt1 Ig domain 2 fused directly to the beginning of VEGFR3 Ig domain 3, with the fusion point defined as TIID of Flt1 (corresponding to amino acids 123-126 of Figure 22A-22C) and continuing into IQLL of VEGFR3 (corresponding to amino acids 127-130 of Figure 22A-22C).

For Ig domain 3 of VEGFR3, the 5' and 3' primers used for RT-PCR were as follows:

5': R3D3.s (ATCCAGCTGTTGCCCAGGAAGTCGCTGGAGCTGCTGGTA)

3': R3D3.as (ATTTTCATGCACAATGACCTCGGTGCTCTCCCGAAATCG)

Both the 5' and 3' amplification primers match the sequence of VEGFR3. The 296bp amplification product of this RT-PCR reaction was isolated by standard techniques and subjected to a second round of PCR to add suitable sequences to allow for fusion of the Flt1D2 with the Flk1D3 domains and fusion of the Flk1D3 and Fc domains via a GPG bridge (see below). The amplification primers were as follows:

5':Flt1D2.VEGFR3D3.s
(TCATAGATATCCAGCTGTTGCCCAGGAAGTCGCTGGAG)

3': VEGFR3D3/srf.as
(GATAATGCCCGGGCCATTTTCATGCACAATGACCTCGGT)

The 5' amplification primer encodes the 3' end of Flt1 Ig domain 2 fused directly to the beginning (5' end) of VEGFR3 Ig domain 3, as described above. The 3' amplification primer encodes the 3' end of VEGFR3 Ig domain 3, defined by the amino acids VIVHEN (corresponding

to amino acids 221-226 of Figure 22A-22C), followed by a bridging sequence that includes a recognition sequence for Srf1, and encodes the amino acids GPG. The bridging sequence corresponds to amino acids 227-229 of Figure 22A-22C.

After one round (for Flt1 Ig domain 2) or two rounds (for Flt4 Ig domain 3) of PCR to produce the individual Ig domains, the PCR products were combined in a tube and subjected to a further round of PCR amplification with the amplification primers bsp/flt1D2 and VEGFR3D3/srf.as described *supra*, to produce the fusion product. This PCR product was subsequently digested with the restriction enzymes BspEI and SmaI and the resulting 625bp fragment was subcloned into the BspEI to SrfI restriction sites of the vector pMT21/Flt1ΔB2.Fc (described *supra*), to create the plasmid pMT21/Flt1D2.VEGFR3D3.Fc. The sequence of the Flt1D2-VEGFR3D3 gene fusion insert was verified by standard sequence analysis. This plasmid was then digested with the restriction enzymes EcoRI and SrfI and the resulting 693bp fragment was subcloned into the EcoRI to SrfI restriction sites of the plasmid pFlt1(1-3)ΔB2-FcΔC1(a) to produce the plasmid designated pFlt1D2.VEGFR3D3.FcΔC1(a). The complete DNA deduced amino acid sequence of the Flt1D2.VEGFR3D3.FcΔC1(a) chimeric molecule is set forth in Figure 22A-22C.

Example 18: Extracellular Matrix Binding (ECM) Binding Assay

ECM-coated plates (Becton Dickinson catalog # 35-4607) were rehydrated with warm DME supplemented with glutamine (2mM), 100U penicillin, 100U streptomycin, and 10% BCS for at least 1 hr. before adding samples. The plates were then incubated for 1 hr. at room temperature with varying concentrations of Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a) starting at 10nM with subsequent 2-fold dilutions in PBS plus 10% BCS. The plates were then washed 3 times with PBS plus 0.1% Triton-X and incubated with alkaline phosphatase-conjugated anti-human Fc antibody (Promega, 1:4000 in PBS plus 10% BCS) for 1 hr. at room temperature. The plates were then washed 4 times with PBS 0.1% Triton-X and alkaline phosphatase buffer/pNPP solution (Sigma) was added for color development. Plates were read at l = 405-570nm. The results of this experiment are shown in Figure 23 and demonstrate that the Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a) proteins are considerably less sticky to the ECM as compared to the Flt1(1-3)-Fc protein.

Example 19: Transient expression of pFlt1D2.Flk1D3.FcΔC1(a) in CHO-K1 (E1A) cells

A large scale (2L) culture of E. coli DH10B cells carrying the pFlt1D2.Flk1D3.FcΔC1(a) plasmid described *supra* in Example 17(a) was grown overnight in Terrific Broth (TB) plus 100μg/ml ampicillin. The

next day, the plasmid DNA was extracted using a QIAgen Endofree Megaprep kit following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined by standard techniques using a UV spectrophotometer and fluorometer. The plasmid DNA was verified by standard restriction enzyme digestion of aliquots using the restriction enzymes EcoRI plus NotI and AseI. All restriction enzyme digest fragments corresponded to the predicted sizes when analyzed on a 1% agarose gel.

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 106 cells/plate. Plating media was Gibco Ham's F-12 supplemented with 10% Hyclone Fetal Bovine Serum (FBS), 100U penicillin/100U streptomycin and glutamine (2mM). The following day each plate of cells was transfected with 6 μg of the pFlt1D2.Flk1D3.FcΔC1(a) plasmid DNA using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells, 12 ml/plate of Optimem supplemented with 10% FBS was added. Plates were incubated at 37°C in a 5% CO_2 incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II supplemented with glutamine (2mM) and 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3 days of incubation, the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet

cells. The supernatant was decanted into sterile 1L bottles and purification of the expressed protein was performed as described *infra*.

Example 20: Construction pVEGFR1R2-FcΔC1(a) expression vector

The pVEGFR1R2.FcΔC1(a) expression plasmid was constructed by insertion of DNA encoding amino acids SDT (corresponding to amino acids 27-29 of Figure 24A-24C) between Flt1d2-Flk1d3-FcΔC1(a) amino acids 26 and 27 of Figure 21A-21C (GG) and removal of DNA encoding amino acids GPG corresponding to amino acids 229-231 of Figure. The SDT amino acid sequence is native to the Flt1 receptor and was added back in to decrease the likelihood of heterogeneous N-terminal processing. The GPG (bridging sequence) was removed so that the Flt1 and Flk1 Ig domains were fused directly to one another. The complete DNA and deduced amino acid sequences of the pVEGFR1R2.FcΔC1(a) chimeric molecule is set forth in Figure 24A-24C.

Example 21: Cell Culture Process Used to Produce Modified Flt1 Receptors

(a) Cell Culture Process Used to Produce Flt1D2.Flk1D3.FcΔC1(a)

The process for production of Flt1D2.Flk1D3.FcΔC1(a) protein using the

expression plasmid pFlt1D2.Flk1D3.FcΔC1(a) described *supra* in Example 1 involves suspension culture of recombinant Chinese hamster ovary (CHO K1/E1A) cells which constitutively express the protein product. The cells are grown in bioreactors and the protein product is isolated and purified by affinity and size exclusion chromatography. The process is provided in greater detail below.

Cell Expansion

Two confluent T-225 cm^2 flasks containing the Flt1D2.Flk1D3.FcΔC1(a) expressing cell line were expanded by passaging cells into eight T-225 cm^2 flasks in medium (GMEM + 10% serum, GIBCO) and incubated at 37°C and 5% CO_2. When the flasks approached confluence (approximately 3 to 4 days) the cells were detached using trypsin. Fresh medium was added to protect the cells from further exposure to the trypsin. The cells were centrifuged and resuspended in fresh medium then transferred to eight 850 cm^2 roller bottles and incubated at 37°C and 5% CO_2 until confluent.

Suspension Culture in Bioreactors

Cells grown in roller bottles were trypsinized to detach them from the surface and washed with suspension culture medium. The cells are aseptically transferred to a 5L bioreactor (New Brunswick Celligen Plus) where the cells are grown in 3.5L of suspension culture. The

suspension culture medium was a glutamine-free low glucose modification of IS-CHO (Irvine Scientific) to which 5% fetal bovine serum (Hyclone), GS supplement (Life Technologies) and 25 μM methionine sulfoximine (Sigma) was added. The pH was controlled at 7.2 by addition of carbon dioxide to the inlet gas or by addition of a liquid solution of sodium carbonate to the bioreactor. Dissolved oxygen level was maintained at 30% of saturation by addition of oxygen or nitrogen to the inlet gas and temperature controlled at 37°C. When a density of 4 $x10^6$ cells/mL was reached the cells were transferred to a 40L bioreactor containing the same medium and setpoints for controlling the bioreactor. The temperature setpoint was reduced to 34°C to slow cell growth and increase the relative rate of protein expression.

(b) Cell Culture Process Used to Produce Flt1D2.VEGFR3D3.FcΔC1(a)

The same methodologies as described *supra* for Flt1D2.Flk1D3.FcΔC1(a) were used to produce Flt1D2.VEGFR3D3.FcΔC1(a).

Example 22: Harvest and Purification of Modified Flt1 Receptors

(a) Harvest and Purification of Flt1D2.Flk1D3.FcΔC1(a)

The product protein was aseptically harvested from the bioreactor while retaining cells using Millipore Prostak tangential-flow filtration modules and a low-shear mechanical pump (Fristam). Fresh medium was added to the bioreactor to replace that removed during the harvest filtration. Approximately 40L of harvest filtrate was then loaded onto a 400 mL column containing Protein A Sepharose resin (Amersham Pharmacia). After loading the resin was washed with buffer containing 10 mM sodium phosphate, 500 mM sodium chloride, pH 7.2 to remove any unbound contaminating proteins. Flt1D2.Flk1D3.FcΔC1(a) protein was eluted with a pH 3.0 citrate buffer. The eluted protein was neutralized by addition of Tris base and frozen at -20°C.

Several frozen lots of Flt1D2.Flk1D3.FcΔC1(a) protein from the Protein A step above were thawed, pooled and concentrated using a Millipore 30kD nominal molecular weight cutoff (NMWCO) tangential flow filtration membrane. The protein was transferred to a stirred cell concentrator (Millipore) and further concentrated to 30 mg/mL using a 30kD NMWCO membrane. The concentrated protein was loaded onto a size exclusion column packed with Superdex 200 resin (Amersham Pharmacia) that was equilibrated with phosphate buffered saline plus 5% glycerol. The same buffer was used to run the column. The fractions corresponding to Flt1D2.Flk1D3.FcΔC1(a) dimer were pooled, sterile filtered through a 0.22 micron filter, aliquoted and frozen.

(b) Harvest and Purification of Flt1D2.VEGFR3D3.FcΔC1(a)

The same methodologies as described *supra* for Flt1D2.Flk1D3.FcΔC1(a) were used to harvest and purify Flt1D2.VEGFR3D3.FcΔC1(a).

Example 23: Phosphorylation Assay for Transiently Expressed VEGFR2

Primary human umbilical vein endothelial cells (HUVECs), passage 4-6, were starved for 2 hrs in serum-free DME high glucose media. Samples containing 40 ng/ml (1nM) human VEGF165, which is a ligand for the VEGF receptors Flt1, Flk1 and Flt4(VEGFR3) were prepared and were preincubated for 1 hr. at room temperature with varying amounts of the modified Flt1 receptors Flt1(1-3)-Fc, Flt1(1-3)-Fc (A40), Flt1D2Flk1D3.FcΔC1(a) and Flt1D2VEGFR3D3.FcΔC1(a) in serum-free DME-high glucose media containing 0.1% BSA. Cells were challenged for 5 minutes with the samples prepared above +/- VEGF165, followed by whole cell lysis using complete lysis buffer. Cell lysates were immunoprecipitated with an antibody directed against the C-terminus of VEGFR2 receptor. The immunoprecipitated lysates were loaded onto 4-12% SDS-PAGE Novex gel and then transferred to PVDF membrane using standard transfer methodologies. Detection of phosphorylated VEGFR2 was done by immunoblotting with the anti-phospho Tyrosine mAb called 4G10 (UBI) and developed using ECL-reagent (Amersham).

Figures 25A-25C and 26A-26B show the results of this experiment. Figure 25A-25C reveals that detection by Western blot of tyrosine phosphorylated VEGFR2(Flk1) by VEGF165 ligand stimulation shows that cell-surface receptors are phosphorylated to varying levels depending on which modified Flt1 receptor is used during the preincubations with VEGF. As is seen in Figure 25A, at a 1.5 molar excess of either Flt1(1-3)-Fc , Flt1(1-3)-Fc (A40) or transient Flt1D2Flk1D3.FcΔC1(a) there is complete blockage of receptor stimulation by these three modified Flt1 receptors as compared to control media challenge. In contrast, transient Flt1D2VEGFR3D3.FcΔC1(a) does not show significant blockage at this molar excess, as compared with VEGF positive control challenge. Similar results are seen in Figure 25B, where the modified Flt receptors are in a 3-fold molar excess to VEGF165 ligand. In Figure 25C, where the modified Flt1 receptors are in a 6-fold molar excess to VEGF165 ligand, transient Flt1D2VEGFR3D3.FcΔC1(a) can now be shown to be partially blocking VEGF165-induced stimulation of cell-surface receptors.

In Figure 26A-26B, detection by Western blot of tyrosine phosphorylated VEGFR2(Flk1) by VEGF165 ligand stimulation shows that cell-surface receptors are not phosphorylated by challenge samples which have VEGF165 preincubated with 1 and 2 fold molar excess (Figure 26A) or 3 and 4 fold molar excess (Figure 26B) of either transient Flt1D2Flk1D3.FcΔC1(a), stable Flt1D2Flk1D3.FcΔC1(a), or transient VEGFR1R2-FcΔC1(a). At all modified Flt1 receptor

concentrations tested there is complete binding of VEGF165 ligand during the preincubation, resulting in no detectable stimulation of cell-surface receptors by unbound VEGF165 as compared to control media challenge.

Example 24: Cell Proliferation Bioassay

The test cell population is MG87 cells that have been stably transfected with a expression plasmid that contains a DNA insert encoding the VEGFR2(Flk1) extracellular domain fused to the TrkB intracellular kinase domain, thus producing a chimeric molecule. The reason the TrkB intracellular kinase domain was used rather than the native VEGFR2(Flk1) intracellular kinase domain is that the intracellular kinase domain of VEGFR2(Flk1) does not cause a strong proliferative response when stimulated by VEGF165 in these cells. It is known that MG87 cells containing full length TrkB receptor give a robust proliferative response when stimulated with BDNF, so the TrkB intracellular kinase domain was engineered to replace the intracellular kinase domain of VEGFR2(Flk1) to take advantage of this proliferative response capability.

5 x 10^3 cells/well were plated in a 96 well plate and allowed to settle for 2 hrs at 37°C. The following modified Flt receptors Flt1(1-3)-Fc, Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a), plus an irrelevant receptor termed Tie2-Fc as a negative control, were titrated

from 40nM to 20pM and incubated on the cells for 1hr at 37°C. Human recombinant VEGF165 in defined media was then added to all the wells at a concentration of 1.56nM. The plates were incubated for 72 hrs at 37°C and then MTS (Owen's reagent, Promega) added and the plates were incubated for an additional for 4 hrs. Finally, the plates were read on a spectrophotometer at 450/570nm. The results of this experiment are shown in Figure 27. The control receptor Tie2-Fc does not block VEGF165-induced cell proliferation at any concentration whereas Flt1D2.Flk1D3.FcΔC1(a) blocks 1.56nM VEGF165 with a half maximal dose of 0.8nM. Flt1(1-3)-Fc and Flt1D2.VEGFR3D3.FcΔC1(a) are less effective in blocking VEGF165 in this assay with a half maximal dose of ~ 2nM. VEGF165 alone gives a reading of 1.2 absorbance units and the background is 0.38 absorbance units.

Example 25: Binding Stoichiometry of Modified Flt Receptors to VEGF165

(a) BIAcore Analysis

The stoichiometry of Flt1D2Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a) interaction with human VEGF165 was determined by measuring either the level of VEGF saturation binding to the Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) surfaces or measuring concentration of VEGF165 needed to completely prevent binding of Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) to VEGF BIAcore chip surface.

Modified Flt receptors Flt1D2Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a), were captured with an anti-Fc specific antibody that was first immobilized on a Biacore chip (BIACORE) using amine-coupling chemistry. A blank antibody surface was used as a negative control. VEGF165 was injected at a concentration of 1 nM, 10 nM, and 50 nM over the Flt1D2Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a) surfaces at 10 μl/min for one hour. A real-time binding signal was recorded and saturation binding was achieved at the end of each injection. Binding stoichiometry was calculated as a molar ratio of bound VEGF165 to the immobilized Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a), using the conversion factor of 1000 RU equivalent to 1 ng/ml. The results indicated binding stoichiometry of one VEGF165 dimeric molecule per one Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) molecule (Figure 28).

In solution, Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) at a concentration of 1nM (estimated to be 1000 times higher than the KD of the Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a)/VEGF165 interaction) were mixed with varied concentrations of VEGF165. After one hour incubation, concentrations of the free Flt1D2Flk1D3.FcΔC1(a) in solution were measured as a binding signal to an amine-coupled VEGF165 surface. A calibration curve was used to convert the Flt1D2Flk1D3.FcΔC1(a) BIAcore binding signal to its molar concentration. The data showed that the addition of 1 nM VEGF165 into

the Flt1D2Flk1D3.FcΔC1(a) solution completely blocked Flt1D2Flk1D3.FcΔC1(a) binding to the VEGF165 surface. This result suggested the binding stoichiometry of one VEGF165 molecule per one Flt1D2Flk1D3.FcΔC1(a) molecule (Figure 29 and Figure 30). When the concentration of Flt1D2Flk1D3.FcΔC1(a) was plotted as a function of added concentration of VEGF165, the slope of the linear portion was -1.06 for Flt1D2Flk1D3.FcΔC1(a) and -1,07 for VEGFR1R2-FcΔC1(a). The magnitude of the slope, very close to negative one, was indicative that one molecule of VEGF165 bound to one molecule of either Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a).

(b) Size Exclusion Chromatography

Flt1D2Flk1D3.FcΔC1(a) was mixed with a 3-fold excess of VEGF165 and the receptor-ligand complex was purified using a Pharmacia Superose 6 size exclusion chromatography column. The receptor-ligand complex was then incubated in a buffer containing 6M guanidine hydrochloride in order to dissociate it into its component proteins. Flt1D2Flk1D3.FcΔC1(a) was separated from VEGF165 using Superose 6 size exclusion chromatography column run in 6M guanidium chloride. In order to determine complex stoichiometry, several injections of Flt1D2Flk1D3.FcΔC1(a) and VEGF165 were made and peak height or peak integrated intensity was plotted as a function of the concentration of injected protein. The calibration was done under condition identical to one used in separating components of Flt1D2Flk1D3.FcΔC1(a)/VEGF

complex. Quantification of the Flt1D2Flk1D3.FcΔC1(a)/VEGF complex composition was based on the calibration curves. The results of this experiment are set forth in Figure 28, which shows the ratio of VEGF165 to Flt1D2Flk1D3.FcΔC1(a) in a complex to be 1:1.

Example 26: Determination of the Binding Stoichiometry of Flt1D2Flk1D3.FcΔC1(a)/VEGF165 Complex by Size Exclusion Chromatography

Flt1D2Flk1D3.FcΔC1(a)/VEGF165 Complex Preparation

VEGF165 (concentration = 3.61 mg/ml) was mixed with CHO cell transiently expressed Flt1D2.Flk1D3.FcΔC1(a) (concentration = 0.9 mg/ml) in molar ratio of 3:1 (VEGF165:Flt1D2.Flk1D3.FcΔC1(a)) and incubated overnight at 4°C.

(a) Size Exclusion Chromatography (SEC) under native conditions

To separate the complex from excess of unbound VEGF165, 50 µl of the complex was loaded on a Pharmacia Superose 12 PC 3.2/30 which was equilibrated in PBS buffer. The sample was eluted with the same buffer at flow rate 40µl/min. at room temperature. The results of this SEC are shown in Figure 31. Peak #1 represents the complex and peak #2 represents unbound VEGF165. Fractions eluted between 1.1 and 1.2

ml were combined and guanidinium hydrochloride (GuHCl)was added to a final concentration 4.5M to dissociate the complex.

(b) Size Exclusion Chromatography (SEC) under dissociative conditions

To separate the components of the receptor-ligand complex and to determine their molar ratio, 50µl of dissociated complex as described *supra* was loaded onto a Superose 12 PC 3.2/30 equilibrated in 6M GuHCl and eluted with the same solution at a flow rate 40µl/min. at room temperature. The results of this SEC are shown in Figure 32. Peak #1 represents Flt1D2Flk1D3.FcΔC1(a) and peak #2 represents VEGF165.

(c) Calculation of Flt1D2Flk1D3.FcΔC1(a):VEGF165 Complex Stoichiometry

The stoichiometry of the receptor-ligand complex was determined from the peak area or the peak height of the components. Concentrations of VEGF165 and Flt1D2Flk1D3.FcΔC1(a) corresponding to the peak height or peak area, respectively, were obtained from the standard curves for VEGF165 and Flt1D2Flk1D3.FcΔC1(a). To obtain a standard curve, four different concentrations (0.04 mg/ml -0.3mg/ml) of either component were injected onto a Pharmacia Superose 12 PC 3.2/30 column equilibrated in 6M guanidinium chloride and eluted with the same

solution at flow rate 40µl/min. at room temperature. The standard curve was obtained by plotting peak area or peak height vs protein concentration. The molar ratio of VEGF165:Flt1D2Flk1D3.FcΔC1(a) determined from the peak area of the components was 1.16. The molar ratio of VEGF165:Flt1D2Flk1D3.FcΔC1(a) determined from the peak height of the components was 1.10.

Example 27: Determination of the Stoichiometry of the Flt1D2Flk1D3.FcΔC1(a)/VEGF165 Complex by Size Exclusion Chromatography with On-Line Light Scattering

Complex preparation

VEGF165 was mixed with CHO transiently expressed Flt1D2.Flk1D3.FcΔC1(a) protein in molar ratio of 3:1 (VEGF165:Flt1D2Flk1D3.FcΔC1(a)) and incubated overnight at 4°C.

(a) Size Exclusion Chromatography (SEC) with On-Line Light Scattering

Size exclusion chromatography column with a MiniDawn on-line light scattering detector (Wyatt Technology, Santa Barbara, California) and refractive index (RI) detectors (Shimadzu, Kyoto, Japan) was used to determine the molecular weight (MW) of the receptor-ligand complex. Samples were injected onto a Superose 12 HR 10/30 column

(Pharmacia) equilibrated in PBS buffer and eluted with the same buffer at flow rate 0.5 ml/min. at room temperature. As shown in Figure 33, the elution profile shows two peaks. Peak #1 represents the receptor-ligand complex and peak #2 represents the unbound VEGF165. MW was calculated from LS and RI signals. The same procedure was used to determine MW of the individual components of the receptor-ligand complex. The results of these determinations are as follows: MW of the Flt1D2Flk1D3.FcΔC1(a)/VEGF165 complex at the peak position is 157 300 (Figure 33), the MW of VEGF165 at the peak position is 44 390 (Figure 34) and the MW of R1R2 at the peak is 113 300 (Figure 35).

These data indicated that the stoichiometry of the Flt1D2Flk1D3.FcΔC1(a)/VEGF complex is 1:1 as its corresponds to the sum of molecular weights for Flt1D2Flk1D3.FcΔC1(a) and VEGF165. Importantly, this method conclusively proved that the Flt1D2Flk1D3.FcΔC1(a)/VEGF165 complex was indeed composed of only one molecule of VEGF165 ligand and only one molecule of the Flt1D2Flk1D3.FcΔC1(a).

Example 28: Peptide Mapping of Flt1D2.Flk1D3.FcΔC1(a)

The disulfide structures and glycosylation sites in Flt1D2.Flk1D3.FcΔC1(a) were determined by a peptide mapping method. In this method, the protein was first cleaved with trypsin. Tryptic fragments were analyzed and identified by HPLC coupled with mass

spectrometry, in addition to an N-terminal sequencing technique. Reduction of the tryptic digest was employed to help identify disulfide-bond-containing fragments. Treatment of the tryptic digest with PNGase F (Glyko, Novato, CA) was employed to help identify fragments with N-linked glycosylation sites. The results are summarized in the accompanying Figure 36.

There are a total of ten cysteines in Flt1D2.Flk1D3.FcΔC1(a); six of them belong to the Fc region. Cys27 has been confirmed to be disulfide bonded to Cys76. Cys121 is confirmed to be disulfide bonded to Cys 182. The first two cysteines in the Fc region (Cys211 and Cys214) form an intermolecular disulfide bond with the same two cysteines in another Fc chain. However, because these two cysteines can not be separated enzymatically from each other, it can not be determined whether disulfide bonding is occurring between same cysteines (Cys211 to Cys211, for example) or between Cys211 and Cys214. Cys216 is confirmed to be disulfide bonded to Cys306. Cys 352 is confirmed to be disulfide bonded to Cys410.

There are five possible N-linked glycosylation sites in Flt1D2.Flk1D3.FcΔC1(a). All five of them are found to be glycosylated to varying degrees. Complete glycosylation was observed at Asn33 (amino acid sequence NIT), Asn193 (amino acid sequence NST), and Asn282 (amino acid sequence NST). In addition, partial glycosylation is

observed on Asn65 and Asn120. Sites of glycosylation are highlighted by underline in the Figure 36.

Example 29: Pharmacokinetic Analysis of Modified Flt Receptors

(a) Pharmacokinetic analysis of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a)

Balb/c mice (25-30g) were injected subcutaneously with 4mg/kg of Flt1(1-3)-Fc (A40), CHO transiently expressed Flt1D2.Flk1D3.FcΔC1(a), CHO stably expressed Flt1D2.Flk1D3.FcΔC1(a), and CHO transiently expressed VEGFR1R2-FcΔC1(a). The mice were tail bled at 1, 2, 4, 6, 24hrs, 2 days, 3 days and 6 days after injection. The sera were assayed in an ELISA designed to detect Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a). The ELISA involves coating an ELISA plate with VEGF165, binding the detect Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) and reporting with an anti-Fc antibody linked to horse radish peroxidase. The results of this experiments are shown in Figure 37. The T_{max} for Flt1(1-3)-Fc (A40) was at 6 hrs while the T_{max} for the transient and stable Flt1D2.Flk1D3.FcΔC1(a) and the transient VEGFR1R2-FcΔC1(a) was 24hrs. The C_{max} for Flt1(1-3)-Fc (A40) was 8μg/ml. For both transients (Flt1D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a)) the C_{max}

was 18μg/ml and the C_{max} for the stable VEGFR1R2-FcΔC1(a) was 30μg/ml.

(b) Pharmacokinetic analysis of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a)

Balb/c mice (25-30g) were injected subcutaneously with 4mg/kg of Flt1(1-3)-Fc (A40), CHO transiently expressed Flt1D2.Flk1D3.FcΔC1(a) and CHO transiently expressed Flt1D2.VEGFR3D3.FcΔC1(a). The mice were tail bled at 1, 2, 5, 6, 7, 8, 12, 15 and 20 days after injection. The sera were assayed in an ELISA designed to detect Flt1(1-3)-Fc, Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a). The ELISA involves coating an ELISA plate with 165, binding the Flt1(1-3)-Fc, Flt1D2.Flk1D3.FcΔC1(a) or Flt1D2.VEGFR3D3.FcΔC1(a) and reporting with an anti-Fc antibody linked to horse radish peroxidase. Flt1(1-3)-Fc (A40) could no longer be detected in the serum after day 5 whereas , Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a) were detectable for 15 days or more. The results of this experiment are shown in Figure 38.

Example 30: Evaluation of the Ability of Flt1D2.Flk1D3.FcΔC1(a) to Inhibit Tumor Growth In Vivo

To evaluate the ability of Flt1D2.Flk1D3.FcΔC1(a) to inhibit tumor growth in vivo a model in which tumor cell suspensions are implanted

subcutaneously on the right flank of male severe combined immunodeficiency (SCID) mice was employed. Two cell lines, the human HT-1080 fibrosarcoma cell line (ATCC accession no. CCL-121) and the rat C6 glioma cell line (ATCC accession no. CCL-107), each of which exhibit distinctly different morphologies and growth characteristics, were used in the assay. The first dose of Flt1D2.Flk1D3.FcΔC1(a) (at 25mg/Kg or as indicated in Figures 39 and 40) was given on the day of tumor implantation. Animals subsequently received subcutaneous injections of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) or vehicle either every other day (EOD) or two times per week (2X/wk) for a period of 2 weeks. After 2 weeks, animals were perfused with fixative, tumors were removed and samples were blinded. Tumor volume was determined by measuring the length and width of visible subcutaneous tumors. Both of Flt1(1-3)-Fc (A40) and Flt1D2.Flk1D3.FcΔC1(a) significantly reduced the growth of tumors formed by HT-1080 and C6 cells. The results of these experiments are shown in Figure 39 and Figure 40.

Example 31: The Effect of VEGF165 and Modified Flt Receptors in Female Reproductive System

The stereotypic pattern of vascular remodeling which occur in the uterus and ovary over the course of the reproductive cycle has been well characterized, making these tissues particularly well suited to the study of mechanisms which regulate angiogenesis, vascular

remodeling and vascular regression. Indeed, *in situ* hybridization studies in the reproductive tissues provided the first clear evidence that VEGF acts as a mediator of physiological angiogenesis in mature rodents, as well as humans and non-human primates (Phillips et al, 1990; Ravindranath et al, 1992; Shweiki et al, 1993; Kamat et al, 1995). As cyclic angiogenesis and vascular remodeling are prominent features of the normal ovary and uterus, it is not surprising that abnormal blood vessel growth and/or vascular dysfunction have been found to characterize many pathological conditions which affect these organs. Furthermore, these pathogenic vascular abnormalities are thought to be caused or perpetuated by the dysregulated expression of one or more angiogenic or anti-angiogenic factors, most prominently VEGF.

For example, abnormal angiogenesis is characteristic of polycystic ovary disease, endometriosis and endometrial carcinoma, and in each case VEGF is over expressed in the affected tissue (Kamat et al, 1995; Shifren et al, 1996; Guidi et al, 1996; Donnez et al, 1998). Overexpression of VEGF is also thought to play a pathogenic role in the establishment of systemic vascular hyperpermeability in ovarian hyperstimulation syndrome (McClure et al, 1994; Levin et al, 1998) and preeclampsia (Baker et al, 1995; Sharkey et al, 1996). In addition, VEGF has been implicated as the permeability factor responsible for the production of ascites associated with ovarian carcinoma and other tumors (Senger et al, 1983; Boocock et al, 1995). Agents which effectively neutralize the biological actions of VEGF can reasonably be

anticipated to be of therapeutic benefit in the above and related conditions.

Angiogenesis and vascular remodeling are also hallmarks of blastocyst implantation and placental development (Findlay, 1986). VEGF is strongly expressed both in the maternal decidua and in embryonic trophoblasts, where it is thought to first stimulate expansion and hyperpermeability of the uterine vasculature during the peri-implantation period and subsequently mediate formation of both the maternal and embryonic components of the placental vasculature (Shweiki et al, 1993; Cullinan-Bove and Koos, 1993; Chakraborty et al, 1995; Das et al, 1997). VEGF is also required for luteal angiogenesis and associated progesterone secretion necessary to prepare the uterus for implantation (Ferrara et al, 1998). Thus, agents which inhibit the biological actions of VEGF may prove to be useful as contraceptive agents (by preventing implantation), or as an abortifacients in the early stages of gestation. The latter application might find particular use as a non-surgical intervention for the termination of ectopic pregnancies.

While the expression of VEGF receptors is largely confined to the vascular endothelium in normal reproductive tissues, Flt1 is also expressed by trophoblasts in the placenta in both humans and animals (Clark et al, 1996; He et al, 1999) where it has been proposed to play a role in trophoblast invasion. Interestingly, both Flt1 and KDR (Flk1) are expressed by choriocarcinoma cell line BeWo (Charnock-Jones et al, 1994), and VEGF has been shown to promote DNA synthesis and tyrosine phosphorylation of MAP kinase in these cells. Furthermore, primary and

metastatic ovarian carcinomas not only to express high levels of VEGF, but - in addition to the vascular endothelium - the tumor cells themselves express KDR and/ or Flt1 (Boocock et al, 1995). These findings suggest that VEGF may not only be critically involved in the generation and maintenance of tumor vasculature, but that at least in some tumors of reproductive origin VEGF may subserve an autocrine role, directly supporting the survival and proliferation of the tumor cells. Thus agents which block the actions of VEGF may have particularly beneficial applications to the treatment of tumors of reproductive origin.

Methods and Results

(a) Assessment of VEGF-Induced Uterine Hyperpermeability

Pregnant mare's serum gonadotrophin (PMSG) was injected subcutaneously (5 IU) to induce ovulation in prepubertal female rats. This results in a surge of estradiol after 2 days which in turn causes an induction of VEGF in the uterus. It is reported that this induction results in hyperpermeability of the uterus and an increase in uterine wet weight 6 hrs. later and, therefore, could potentially be blocked by the modified Flt receptors Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a). In this in vivo model, the normal weight of the rat uterus is about 50 mg and this can be induced to 300-350 mg by PMSG. Desiccation of the tissue reveals that this is all water weight. Subcutaneous injection of Flt1(1-3)-Fc (A40),

Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a) at 25mg/kg at 1hr. after PMSG injection results in about a 50% inhibition of the increase in uterine wet weight. Increasing the dose of modified Flt receptor does not further reduce the increase in wet weight suggesting that there is a VEGF-independent component to this model. The results of this experiment are shown in Figure 41.

(a) Assessment of corpus luteum angiogenesis using progesterone as a readout

Pregnant mare's serum gonadotrophin (PMSG) is injected subcutaneously (5 IU) to induce ovulation in prepubertal female rats. This results in a fully functioning corpus luteum containing a dense network of blood vessels after 4 days that allows for the secretion of progesterone into the blood stream in order to prepare the uterus for implantation. The induction of angiogenesis in the corpus luteum requires VEGF; therefore, blocking VEGF would result in a lack of new blood vessels and thus a lack of progesterone secreted into the blood stream. In this in vivo model, resting levels of progesterone are about 5ng/ml and this can be induced to a level of 25-40ng/ml after PMSG. Subcutaneous injection of Flt1(1-3)-Fc (A40) or Flt1D2.Flk1D3.FcΔC1(a) at 25mg/kg or 5mg/kg at 1hr. after PMSG injection results in a complete inhibition of the progesterone induction on day 4. The results of this experiment are shown in Figure 42A-42B.

Example 33: Pharmacokinetic Analysis of Flt1(1-3)-Fc (A40) and Pegylated Flt1(1-3)-Fc

Flt1(1-3)-Fc was PEGylated with either 10kD PEG or 20kD PEG and tested in balb/c mice for their pharmacokinetic profile. Both PEGylated forms of Flt1(1-3)-Fc were found to have much better PK profiles than Flt1(1-3)-Fc (A40), with the Tmax occurring at 24 hrs. for the PEGylated molecules as opposed to 6 hrs. for Flt1(1-3)-Fc (A40).

Example 34: VEGF165 ELISA to Test Affinity of Modified Flt1 Receptor Variants

10pM of VEGF165 was incubated overnight at room temperature with modified Flt1 receptor variants ranging from 160pM to 0.1pM. The modified Flt1 receptor variants used in this experiment were Flt1(1-3)-Fc, Flt1(1-3)-Fc (A40), transiently expressed Flt1D2Flk1D3.FcΔC1(a), transiently expressed Flt1D2VEFGFR3D3-FcΔC1(a), Flt1-(1-3_{NAS})-Fc, Flt1(1-$3_{R->C}$)-Fc and Tie2-Fc. Flt1(1-3_{NAS})-Fc is a modified version of Flt1(1-3)-Fc in which the highly basic amino acid sequence KNKRASVRRR is replaced by NASVNGSR, resulting in the incorporation of two new glycosylation sites and a net reduction of five positive charges, both with the purpose of reducing the unfavorable effects of this sequence on PK. Flt1(1-$3_{R->C}$)-Fc is a modification in which a single arginine (R) residue within the same basic amino acid sequence is changed to a cysteine (C) (KNKRASVRRR ->

KNKCASVRRR) to allow for pegylation at that residue, which could then shield the basic region from exerting its unfavorable effects on PK. After incubation the solution was transferred to a plate containing a capture antibody for VEGF165 (R&D). The amount of free VEGF165 was then determined using an antibody to report free VEGF165. This showed that the modified Flt1 receptor variant with the highest affinity for VEGF165 (determined as the lowest amount of free VEGF165) was Flt1D2Flk1D3.FcΔC1(a), followed by Flt1(1-3)-Fc and Flt1(1-3)-Fc (A40) and then by Flt1(1-$3_{R->C}$)-Fc, Flt1(1-3_{NAS})-Fc and Flt1D2VEFGFR3D3-FcΔC1(a). Tie2Fc has no affinity for VEGF165.

WE CLAIM:

1. An isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a VEGF polypeptide comprising:

(a) a nucleotide sequence encoding a VEGF receptor component operatively linked to

(b) a nucleotide sequence encoding a multimerizing component, wherein the VEGF receptor component is the only VEGF receptor component of the fusion polypeptide and wherein the nucleotide sequence of (a) consists essentially of a nucleotide sequence encoding the amino acid sequence of Ig domain 2 of the extracellular domain of a first VEGF receptor and a nucleotide sequence encoding the amino acid sequence of Ig domain 3 of the extracellular domain of a second VEGF receptor.

2. The isolated nucleic acid of claim 1 wherein the first VEGF receptor is Flt1.

3. The isolated nucleic acid of claim 1 wherein the second VEGF receptor is Flk1.

4. The isolated nucleic acid of claim 1 wherein the second VEGF receptor is Flt4.

5. The isolated nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding Ig domain 2 of the extracellular domain of the first VEGF receptor is upstream of the nucleotide sequence encoding Ig domain 3 of the extracellular domain of the second VEGF receptor.

6. The isolated nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding Ig domain 2 of the extracellular domain of the first VEGF receptor is downstream of the nucleotide sequence encoding Ig domain 3 of the extracellular domain of the second VEGF receptor.

7. The isolated nucleic acid molecule of claim 1, wherein the multimerizing component comprises an immunoglobulin domain.

8. The isolated nucleic acid molecule of claim 1, wherein the immunoglobulin domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

9. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a modified Flt1 receptor fusion polypeptide, wherein the coding region of the nucleic acid molecule consists of a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence set forth in Figure 13A-13D;

(b) the nucleotide sequence set forth in Figure 14A-14C;

(c) the nucleotide sequence set forth in Figure 15A-15C;

(d) the nucleotide sequence set forth in Figure 16A-16D;

(e) the nucleotide sequence set forth in Figure 21A-21C

(f) the nucleotide sequence set forth in Figure 22A-22C;

(g) the nucleotide sequence set forth in Figure 24A-24C; and

(h) a nucleotide sequence which, as a result of the degeneracy of the genetic code, differs from the nucleotide sequence of (a), (b), (c), (d), (e), (f), or (g) and which encodes a fusion polypeptide molecule having the biological activity of the modified Flt1 receptor fusion polypeptide.

10. A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 1, 2, 3, 4 or 9.

11. A composition capable of binding a VEGF molecule to form a nonfunctional complex comprising a multimer of the fusion polypeptide of claim 10.

12. The composition of claim 11, wherein the multimer is a dimer.

13. The composition of claim 12 and a carrier.

14. A vector which comprises the nucleic acid molecule of claim 1, 2, 3, 4 or 9.

15. An expression vector comprising a nucleic acid molecule of claim 1, 2, 3, 4 or 9 wherein the nucleic acid molecule is operatively linked to an expression control sequence.

16. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 15, in a suitable host cell.

17. The host-vector system of claim 16, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell, or mammalian cell.

18. The host-vector system of claim 16, wherein the suitable host cell is E. coli.

19. The host-vector system of claim 16, wherein the suitable host cell is a COS cell.

20. The host-vector system of claim 14, wherein the suitable host cell is a CHO cell.

21. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 16, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

22. A fusion polypeptide encoded by the nucleic acid sequence set forth in Figure 10A-10D or Figure 24A-24C, which has been modified by acetylation or pegylation.

23. The fusion polypeptide of claim 22 wherein the modification is acetylation.

24. The fusion polypeptide of claim 22 wherein the modification is pegylation.

25. The fusion polypeptide of claim 23 wherein the acetylation is accomplished with at least about a 100 fold molar excess of acetylation reagent.

26. The fusion polypeptide of claim 23 wherein acetylation is accomplished with a molar excess of acetylation reagent ranging from at least about a 10 fold molar excess to about a 100 fold molar excess.

27. The fusion polypeptide of claim 24 wherein the pegylation is 10K or 20K PEG.

28. A method of decreasing or inhibiting plasma leakage in a mammal comprising administering to the mammal fusion polypeptide of claim 10.

29. The method of claim 28, wherein the mammal is a human.

30. The method of claim 29, wherein the fusion polypeptide is acetylated.

31. The method of claim 29, wherein the fusion polypeptide is pegylated.

32. The fusion polypeptide of claims 10 which specifically binds the VEGF receptor ligand VEGF.

33. A method of blocking blood vessel growth in a human comprising administering an effective amount of the fusion polypeptide of claim 10.

34. A method of inhibiting VEGF receptor ligand activity in a mammal comprising administering to the mammal an effective amount of the fusion polypeptide of claim 10.

35. The method of claim 34, wherein the mammal is a human.

36. The method of claim 34, used to attenuate or prevent tumor growth in a human.

37. The method of claim 34, used to attenuate or prevent edema in a human.

38. The method of claim 34, used to attenuate or prevent ascites formation in a human.

39. The method of claim 37, wherein the edema is brain edema.

40. The method of claim 38, wherein the ascites is ovarian cancer associated ascites.

41. A fusion polypeptide capable of binding a VEGF polypeptide comprising:

(a a VEGF receptor component operatively linked to

(b) a multimerizing component,

wherein the VEGF receptor component is the only VEGF receptor component in the fusion polypeptide and consists essentially of the amino acid sequence of Ig domain 2 of the extracellular domain of a first VEGF receptor and the amino acid sequence of Ig domain 3 of the extracellular domain of a second VEGF receptor.

42. The fusion polypeptide of claim 41 wherein the first VEGF receptor is Flt1.

43. The fusion polypeptide of claim 41 wherein the second VEGF receptor is Flk1.

44. The fusion polypeptide of claim 41 wherein the second VEGF receptor is Flt4.

45. The fusion polypeptide claim 41, wherein amino acid sequence of Ig domain 2 of the extracellular domain of the first VEGF receptor is upstream of the amino acid sequence of Ig domain 3 of the extracellular domain of the second VEGF receptor.

46. The fusion polypeptide of claim 41, wherein the amino acid sequence of Ig domain 2 of the extracellular domain of the first VEGF receptor is downstream of the amino acid sequence of Ig domain 3 of the extracellular domain of the second VEGF receptor.

47. The fusion polypeptide of claim 41, wherein the multimerizing component comprises an immunoglobulin domain.

48. The fusion polypeptide of claim 41, wherein the immunoglobulin domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

49. An fusion polypeptide comprising an amino acid sequence of a modified Flt1 receptor, wherein the amino acid sequence selected from

the group consisting of:

(a) the amino acid sequence set forth in Figure 13A-13D;

(b) the amino acid sequence set forth in Figure 14A-14C;

(c) the amino acid sequence set forth in Figure 15A-15C;

(d) the amino acid sequence set forth in Figure 16A-16D;

(e) the amino acid sequence set forth in Figure 21A-21C

(f) the amino acid sequence set forth in Figure 22A-22C; and

(g) the amino acid sequence set forth in Figure 24A-24C.

50. A method of decreasing or inhibiting plasma leakage in a mammal comprising administering to the mammal fusion polypeptide of claim 41, 42, 43, 44 or 49.

51. A method of inhibiting VEGF receptor ligand activity in a mammal comprising administering to the mammal an effective amount of the fusion polypeptide of claim 41, 42, 43, 44 or 49.

Fig.1.

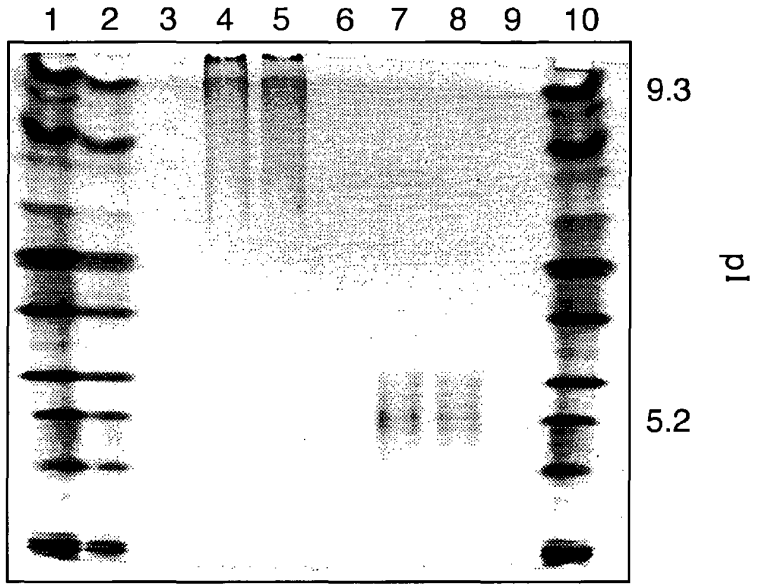

1 2 3 4 5 6 7 8 9 10
9.3
5.2
pI

Fig.2.

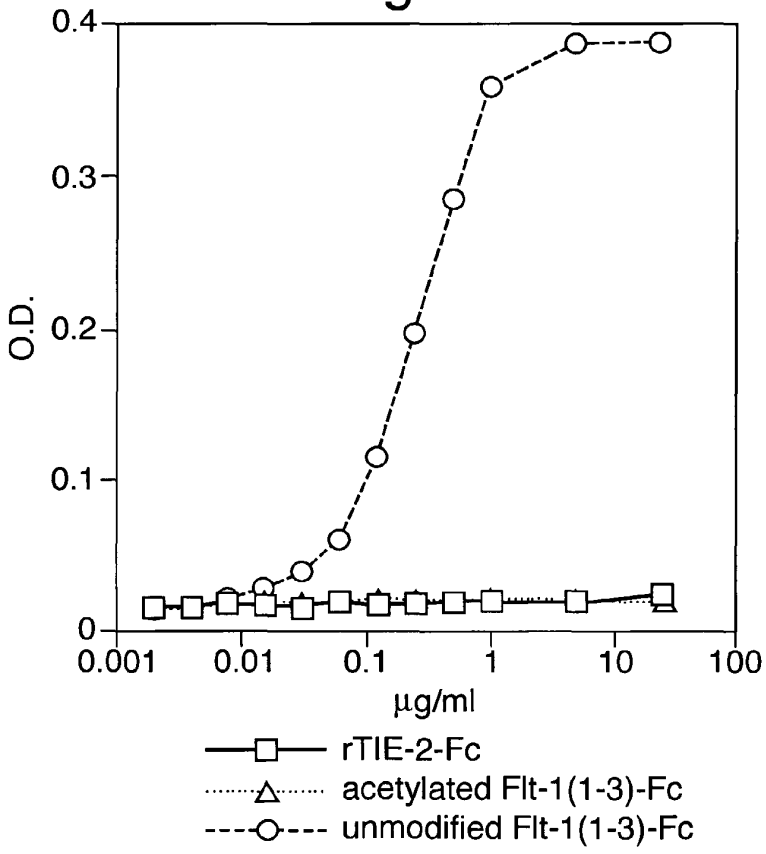

0.4
0.3
0.2
0.1
0
O.D.
0.001
0.01
0.1
1
10
100
µg/ml
rTIE-2-Fc
acetylated Flt-1(1-3)-Fc
unmodified Flt-1(1-3)-Fc

Fig.3.

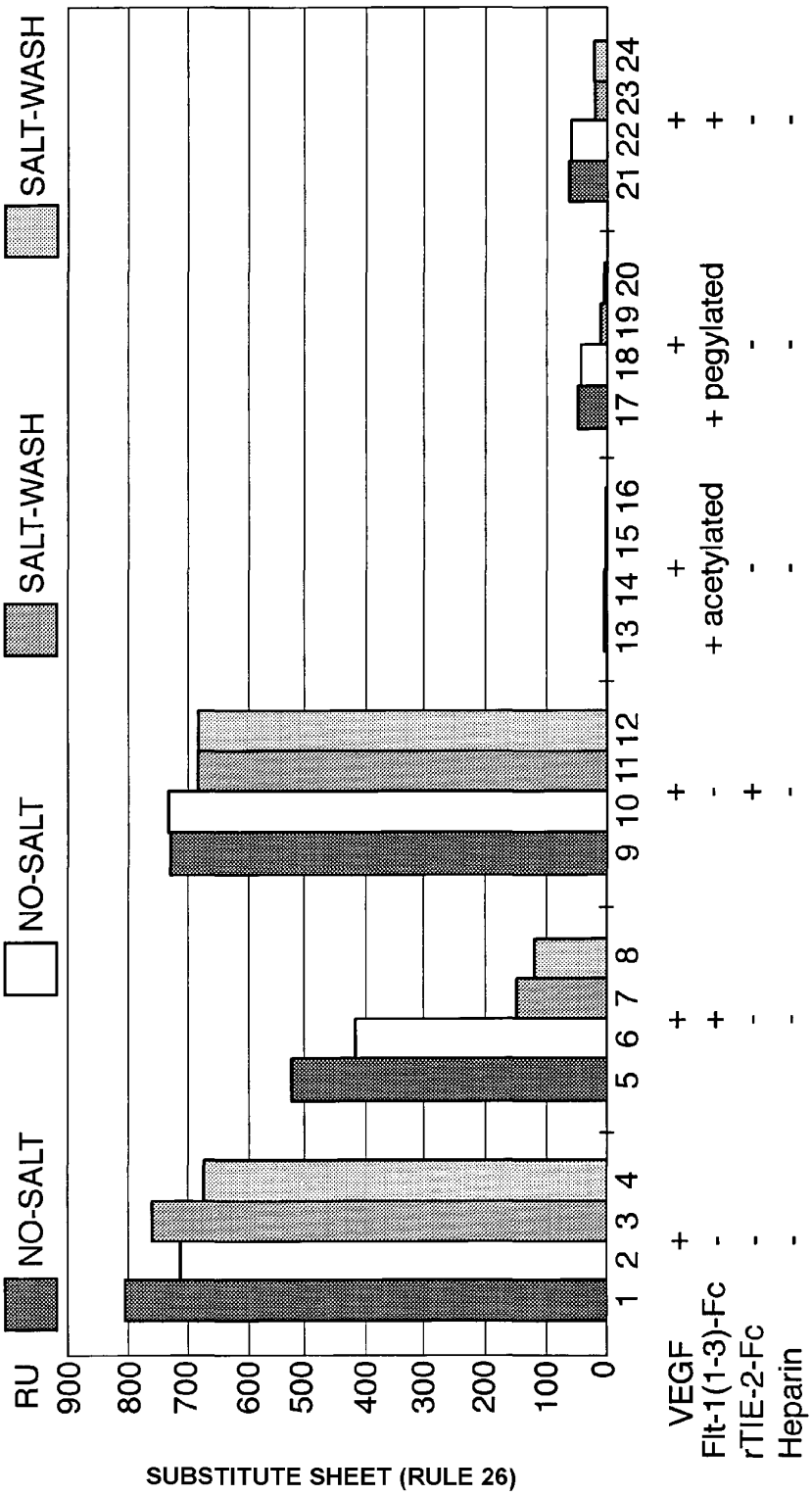

NO-SALT
NO-SALT
SALT-WASH
SALT-WASH
RU
900
800
700
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
VEGF +
Flt-1(1-3)-Fc - + - + - + - + - + - + - + - + - + - + - + - +
rTIE-2-Fc - - + + - - + + - - + + - - + + - - + + - - + +
Heparin - - - - + + + + - - - - + + + + - - - - + + + +
+ acetylated
+ pegylated

Fig.4.

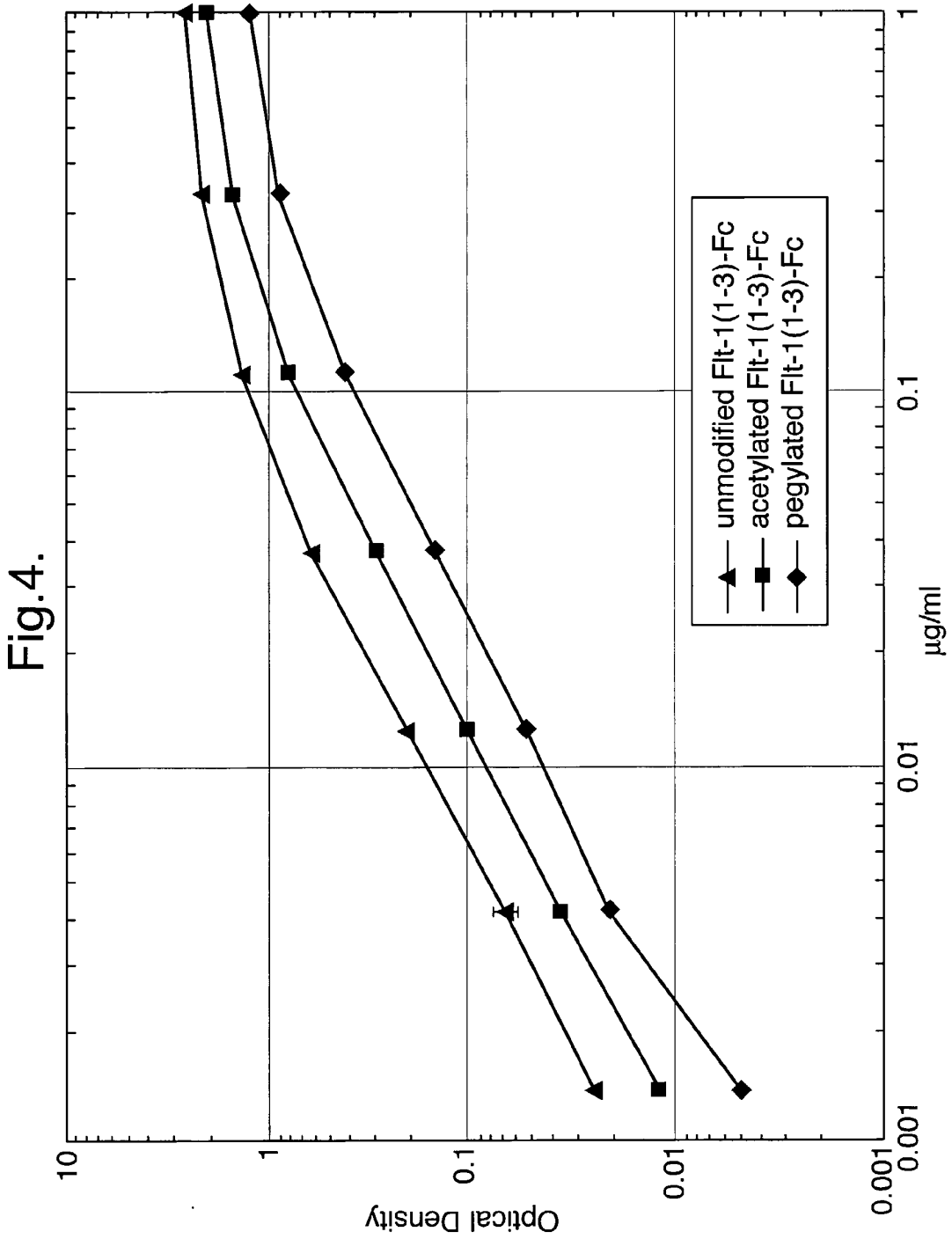

unmodified Flt-1(1-3)-Fc
acetylated Flt-1(1-3)-Fc
pegylated Flt-1(1-3)-Fc
Optical Density
10
1
0.1
0.01
0.001
0.001
0.01
0.1
1
µg/ml

Fig.5.

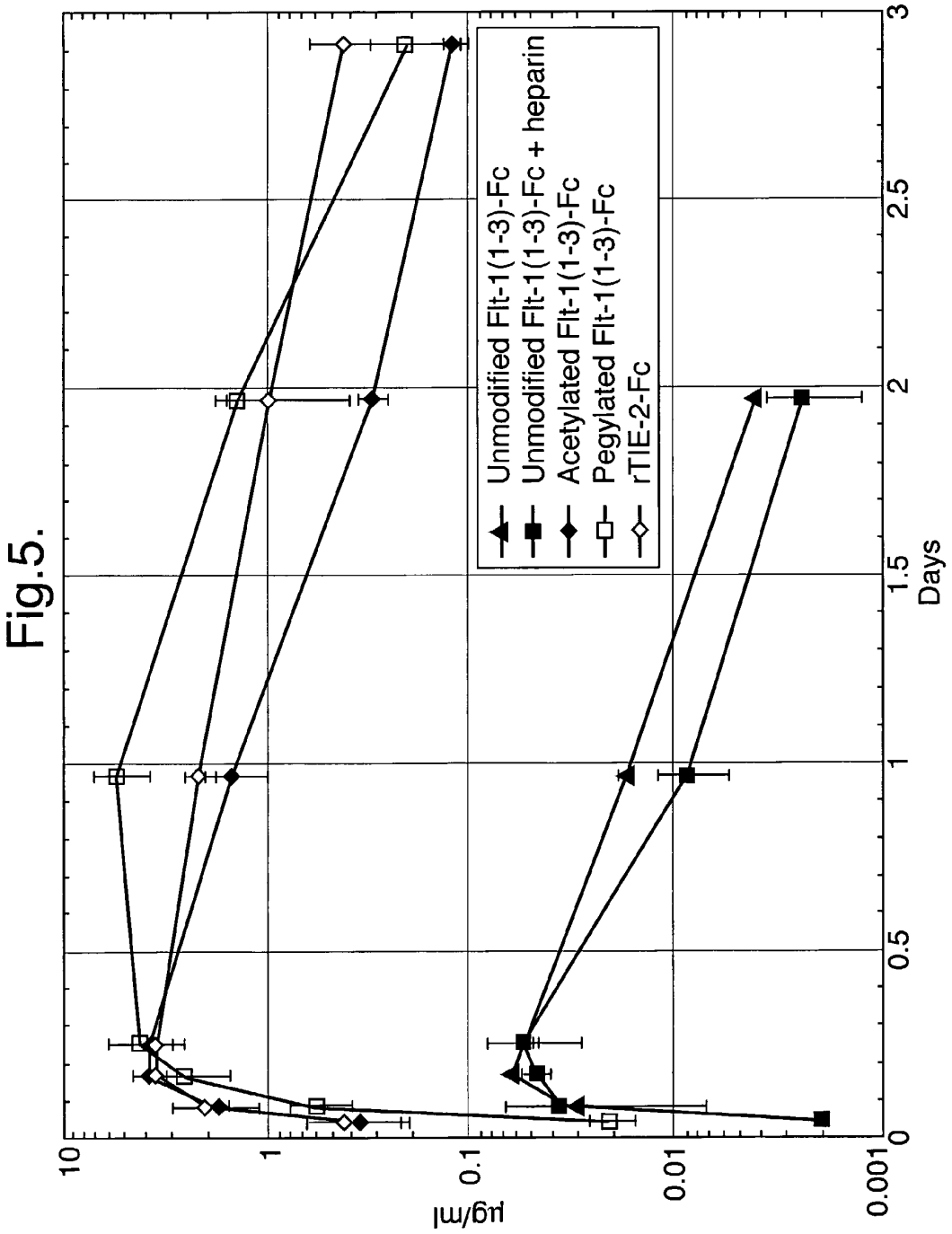

Unmodified Flt-1(1-3)-Fc
Unmodified Flt-1(1-3)-Fc + heparin
Acetylated Flt-1(1-3)-Fc
Pegylated Flt-1(1-3)-Fc
rTIE-2-Fc
μg/ml
10
1
0.1
0.01
0.001
0
0.5
1
1.5
2
2.5
3
Days

Fig.6A.

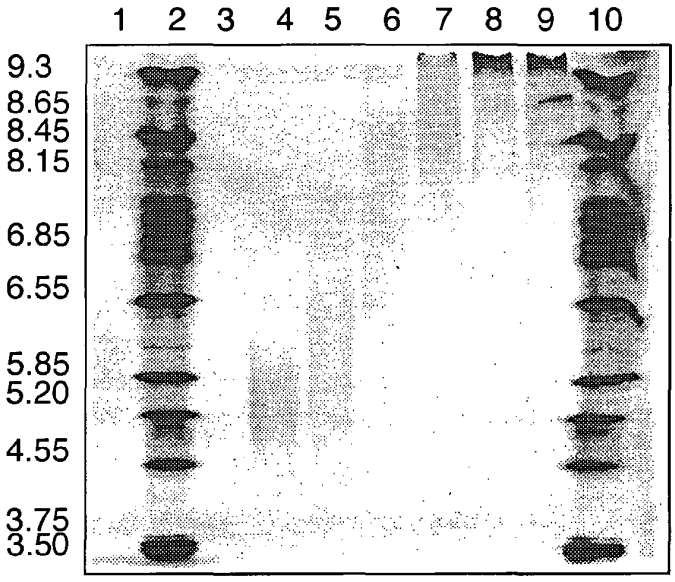

1 2 3 4 5 6 7 8 9 10
9.3
8.65
8.45
8.15
6.85
6.55
5.85
5.20
4.55
3.75
3.50

Fig.6B.

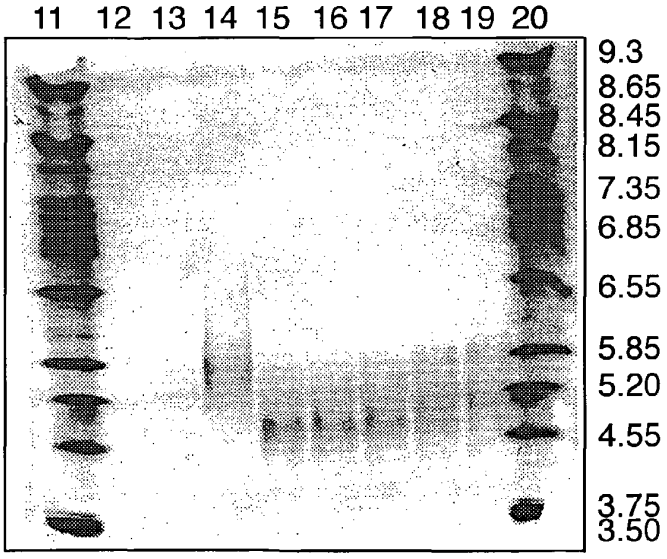

11 12 13 14 15 16 17 18 19 20
9.3
8.65
8.45
8.15
7.35
6.85
6.55
5.85
5.20
4.55
3.75
3.50

Fig.7.

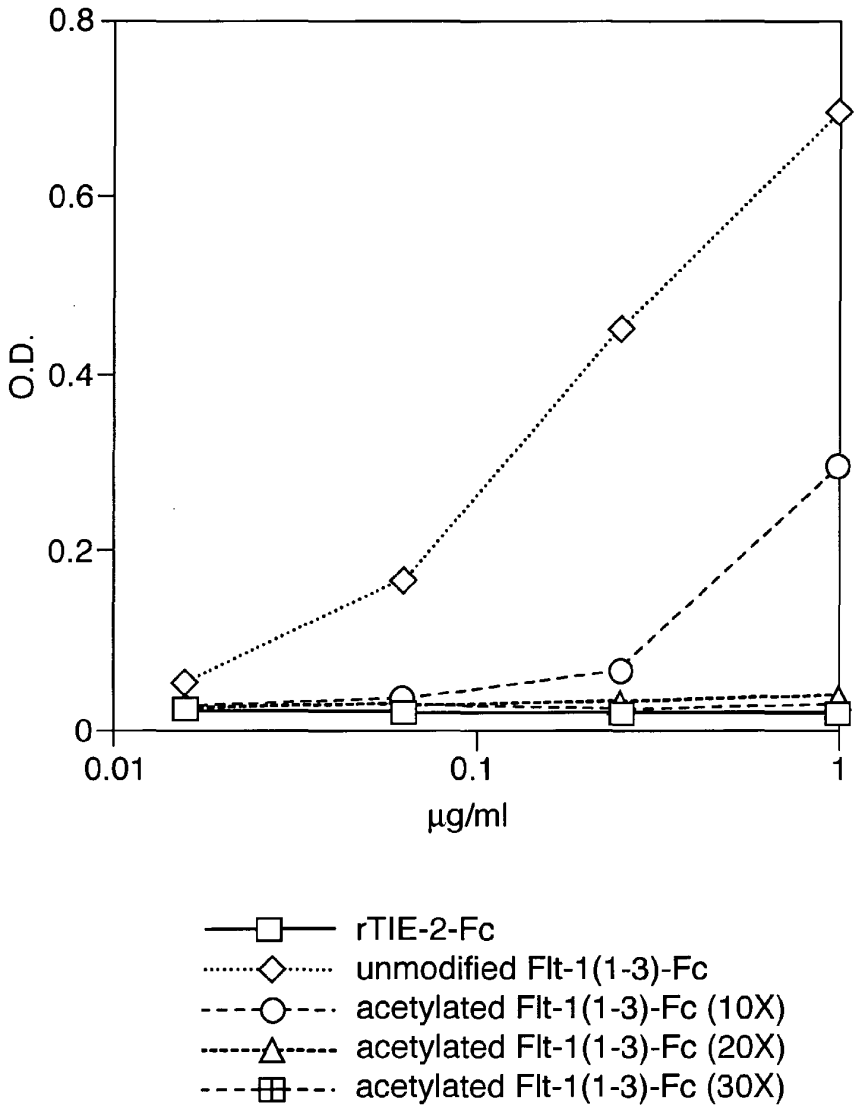
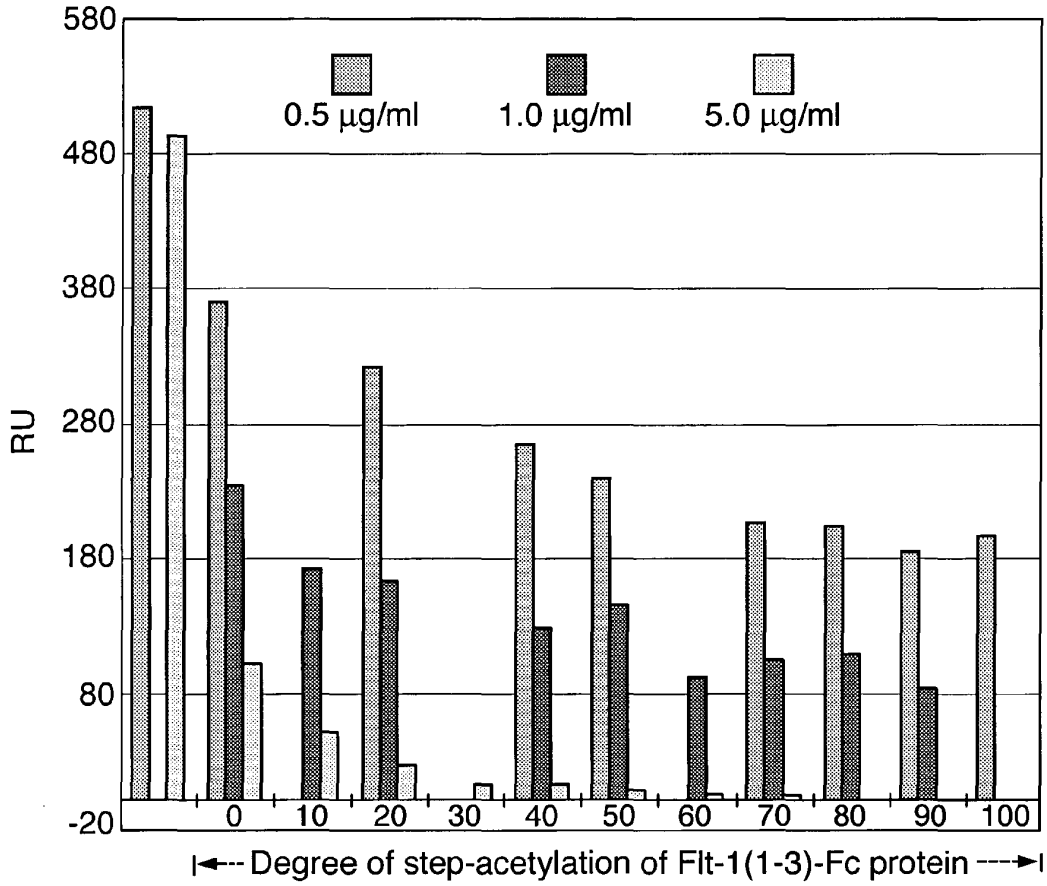

0.8
0.6
0.4
0.2
0
O.D.
0.01
0.1
1
µg/ml
rTIE-2-Fc
unmodified Flt-1(1-3)-Fc
acetylated Flt-1(1-3)-Fc (10X)
acetylated Flt-1(1-3)-Fc (20X)
acetylated Flt-1(1-3)-Fc (30X)

Fig.8.



VEGF 0.2 µg/ml	+	+	+	+	+	+	+	+	+	+	+	+
Unmodified Flt-1(1-3) -Fc	-	+	-	-	-	-	-	-	-	-	-	-
Acetylated Flt-1(1-3) -Fc	-	-	+	+	+	+	+	+	+	+	+	+

Fig.9.

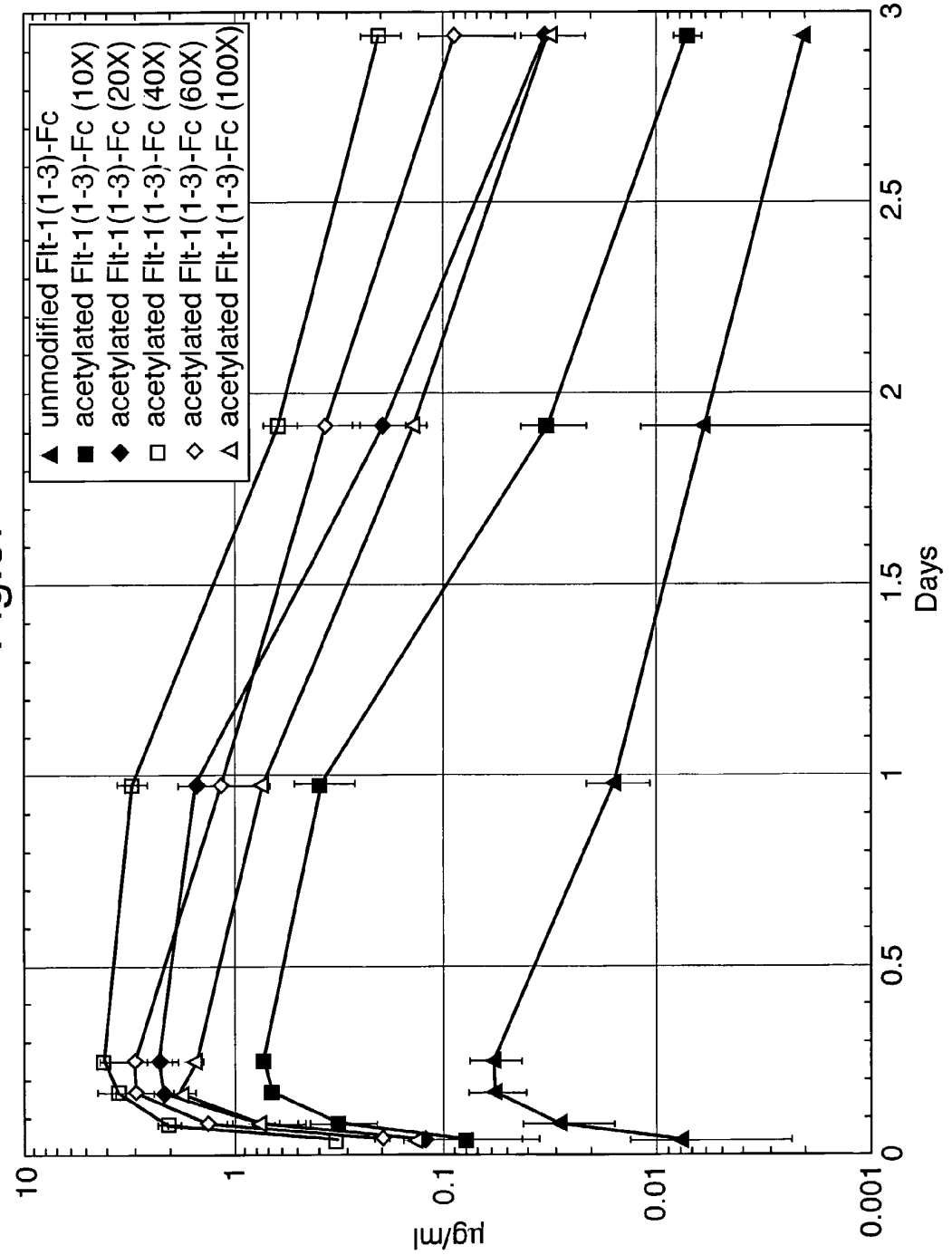

unmodified Flt-1(1-3)-Fc
acetylated Flt-1(1-3)-Fc (10X)
acetylated Flt-1(1-3)-Fc (20X)
acetylated Flt-1(1-3)-Fc (40X)
acetylated Flt-1(1-3)-Fc (60X)
acetylated Flt-1(1-3)-Fc (100X)
μg/ml
10
1
0.1
0.01
0.001
0
0.5
1
1.5
2
2.5
3
Days

Fig.10A.

```
          10          20          30          40          50          60
     *    *     *     *     *     *     *     *     *     *     *     *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG CGC GAC GAG TCG ACA GAC GAA GAG
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu>

          70          80          90          100         110         120
     *    *     *     *     *     *     *     *     *     *     *     *
ACA GGA TCT AGT TCA GGT TCA AAA TTA AAA GAT CCT GAA CTG AGT TTA AAA GGC ACC CAG
TGT CCT AGA TCA AGT CCA AGT TTT AAT TTT CTA GGA CTT GAC TCA AAT TTT CCG TGG GTC
Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln>

          130         140         150         160         170         180
     *    *     *     *     *     *     *     *     *     *     *     *
CAC ATC ATG CAA GCA GGC CAG ACA CTG CAT CTC CAA TGC AGG GGG GAA GCA GCC CAT AAA
GTG TAG TAC GTT CGT CCG GTC TGT GAC GTA GAG GTT ACG TCC CCC CTT CGT CGG GTA TTT
His Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys>

          190         200         210         220         230         240
     *    *     *     *     *     *     *     *     *     *     *     *
TGG TCT TTG CCT GAA ATG GTG AGT AAG GAA AGC GAA AGG CTG AGC ATA ACT AAA TCT GCC
ACC AGA AAC GGA CTT TAC CAC TCA TTC CTT TCG CTT TCC GAC TCG TAT TGA TTT AGA CGG
Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala>

          250         260         270         280         290         300
     *    *     *     *     *     *     *     *     *     *     *     *
TGT GGA AGA AAT GGC AAA CAA TTC TGC AGT ACT TTA ACC TTG AAC ACA GCT CAA GCA AAC
ACA CCT TCT TTA CCG TTT GTT AAG ACG TCA TGA AAT TGG AAC TTG TGT CGA GTT CGT TTG
Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn>

          310         320         330         340         350         360
     *    *     *     *     *     *     *     *     *     *     *     *
CAC ACT GGC TTC TAC AGC TGC AAA TAT CTA GCT GTA CCT ACT TCA AAG AAG AAG GAA ACA
GTG TGA CCG AAG ATG TCG ACG TTT ATA GAT CGA CAT GGA TGA AGT TTC TTC TTC CTT TGT
His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys Lys Lys Glu Thr>

          370         380         390         400         410         420
     *    *     *     *     *     *     *     *     *     *     *     *
GAA TCT GCA ATC TAT ATA TTT ATT AGT GAT ACA GGT AGA CCT TTC GTA GAG ATG TAC AGT
CTT AGA CGT TAG ATA TAT AAA TAA TCA CTA TGT CCA TCT GGA AAG CAT CTC TAC ATG TCA
Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser>

          430         440         450         460         470         480
     *    *     *     *     *     *     *     *     *     *     *     *
GAA ATC CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT
CTT TAG GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA
Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val>

          490         500         510         520         530         540
     *    *     *     *     *     *     *     *     *     *     *     *
ACG TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT
TGC AGT GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA
Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp>
```

Fig.10B.

```
          550         560         570         580         590         600
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA
CCT TTT GCG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT
Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys>

          610         620         630         640         650         660
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
GAA ATA GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT
CTT TAT CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA
Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr>

          670         680         690         700         710         720
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
CTC ACA CAT CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA CCA CGC CCA GTC
GAG TGT GTA GCT GTT TGG TTA TGT TAG TAT CTA CAG GTT TAT TCG TGT GGT GCG GGT CAG
Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val>

          730         740         750         760         770         780
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
AAA TTA CTT AGA GGC CAT ACT CTT GTC CTC AAT TGT ACT GCT ACC ACT CCC TTG AAC ACG
TTT AAT GAA TCT CCG GTA TGA GAA CAG GAG TTA ACA TGA CGA TGG TGA GGG AAC TTG TGC
Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr>

          790         800         810         820         830         840
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
AGA GTT CAA ATG ACC TGG AGT TAC CCT GAT GAA AAA AAT AAG AGA GCT TCC GTA AGG CGA
TCT CAA GTT TAC TGG ACC TCA ATG GGA CTA CTT TTT TTA TTC TCT CGA AGG CAT TCC GCT
Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg>

          850         860         870         880         890         900
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
CGA ATT GAC CAA AGC AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT CTT ACT ATT GAC AAA
GCT TAA CTG GTT TCG TTA AGG GTA CGG TTG TAT AAG ATG TCA CAA GAA TGA TAA CTG TTT
Arg Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys>

          910         920         930         940         950         960
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT TGT CGT GTA AGG AGT GGA CCA TCA TTC AAA
TAC GTC TTG TTT CTG TTT CCT GAA ATA TGA ACA GCA CAT TCC TCA CCT GGT AGT AAG TTT
Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys>

          970         980         990        1000        1010        1020
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
TCT GTT AAC ACC TCA GTG CAT ATA TAT GAT AAA GCA GGC CCG GGC GAG CCC AAA TCT TGT
AGA CAA TTG TGG AGT CAC GTA TAT ATA CTA TTT CGT CCG GGC CCG CTC GGG TTT AGA ACA
Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Gly Pro Gly Glu Pro Lys Ser Cys>

         1030        1040        1050        1060        1070        1080
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC
CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val>
```

Fig.10C.

```
        1090        1100        1110        1120        1130        1140
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA
AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr>

        1150        1160        1170        1180        1190        1200
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC
ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp>

        1210        1220        1230        1240        1250        1260
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC
CCG CAC CTC CAC GTA TTA CGG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr>

        1270        1280        1290        1300        1310        1320
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG
GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys>

        1330        1340        1350        1360        1370        1380
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys>

        1390        1400        1410        1420        1430        1440
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG
CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC GGG GGT AGG GCC CTA CTC GAC TGG TTC
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys>

        1450        1460        1470        1480        1490        1500
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG
TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu>

        1510        1520        1530        1540        1550        1560
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC
ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser>

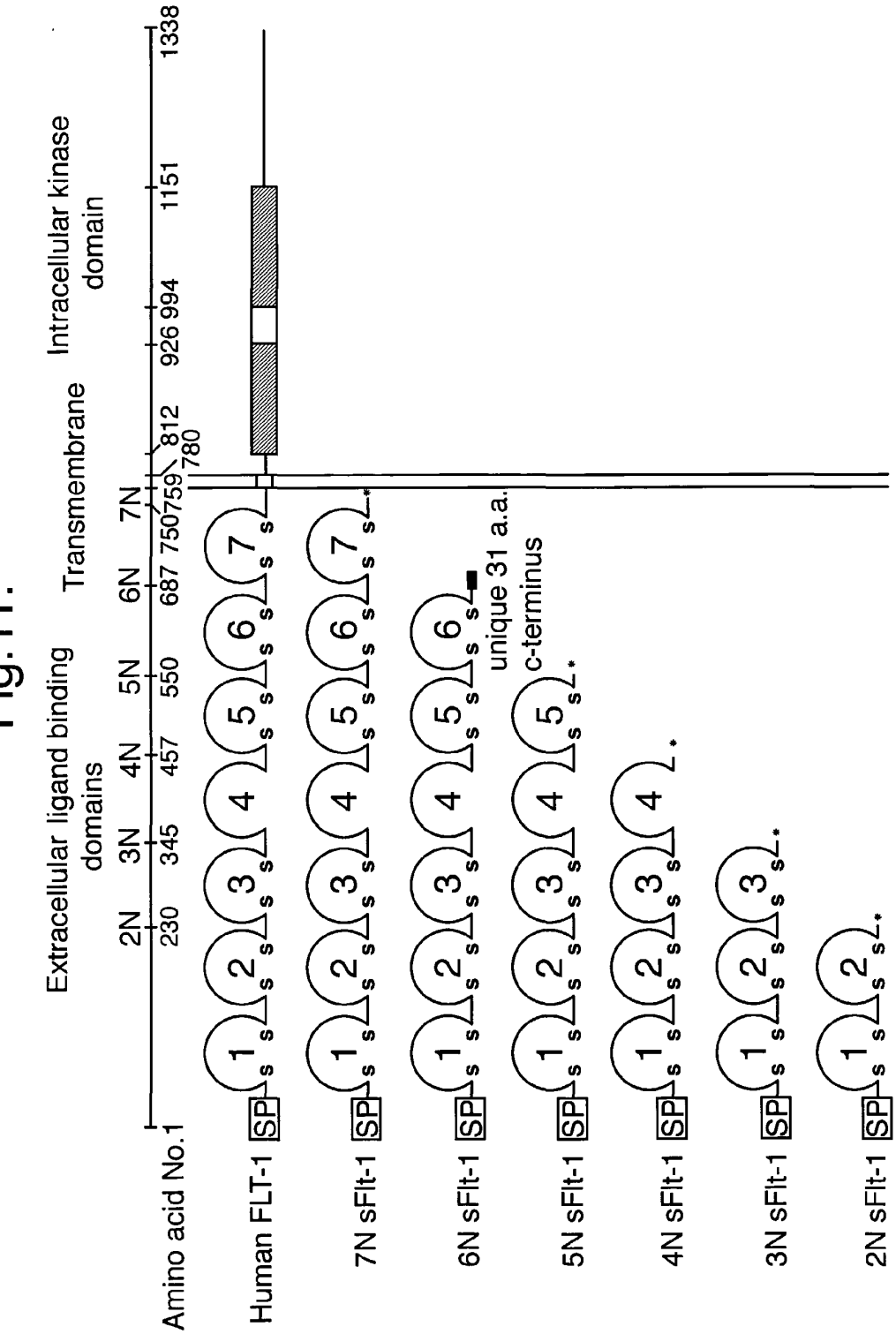
        1570        1580        1590        1600        1610        1620
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG
CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly>
```

Fig.10D.

```
        1630         1640         1650         1660         1670         1680
     *      *     *      *      *     *      *      *     *      *      *     *
AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC
TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser>

        1690         1700
     *      *     *      *
CTC TCC CTG TCT CCG GGT AAA TGA
GAG AGG GAC AGA GGC CCA TTT ACT
Leu Ser Leu Ser Pro Gly Lys ***>
```

Fig.11.


Extracellular ligand binding domains
Transmembrane
Intracellular kinase domain
2N
3N
4N
5N
6N
7N
Amino acid No.1
230
345
457
550
687
750
759
780
812
926
994
1151
1338
Human FLT-1
7N sFlt-1
6N sFlt-1
5N sFlt-1
4N sFlt-1
3N sFlt-1
2N sFlt-1
SP
unique 31 a.a.
c-terminus

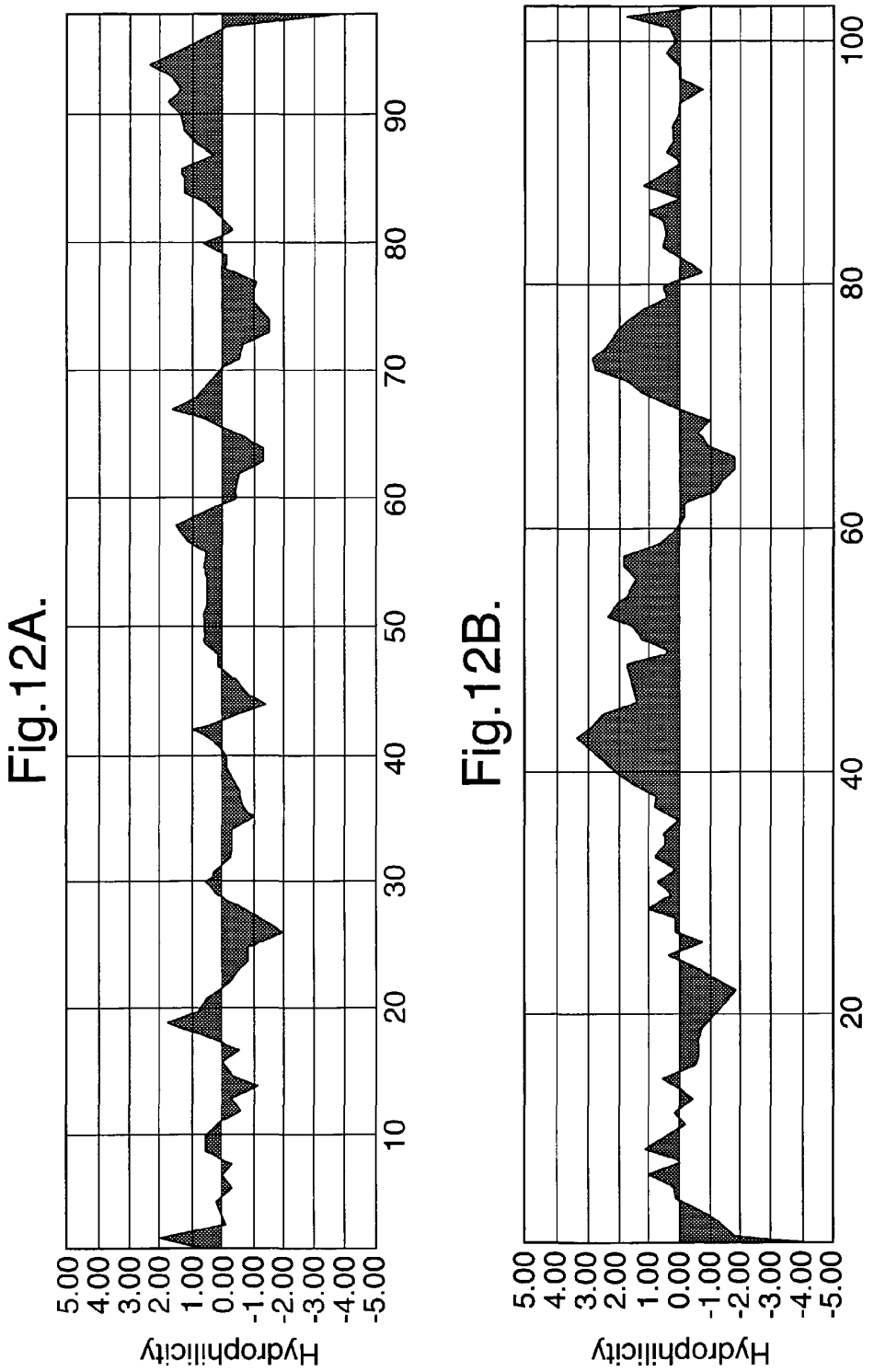

Fig.12A.
Hydrophilicity
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
10
20
30
40
50
60
70
80
90
Fig.12B.
Hydrophilicity
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
20
40
60
80
100

Fig.13A.

```
          10          20          30          40          50          60
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG CGC GAC GAG TCG ACA GAC GAA GAG
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu>

          70          80          90          100         110         120
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
ACA GGA TCT AGT TCA GGT TCA AAA TTA AAA GAT CCT GAA CTG AGT TTA AAA GGC ACC CAG
TGT CCT AGA TCA AGT CCA AGT TTT AAT TTT CTA GGA CTT GAC TCA AAT TTT CCG TGG GTC
Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln>

          130         140         150         160         170         180
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
CAC ATC ATG CAA GCA GGC CAG ACA CTG CAT CTC CAA TGC AGG GGG GAA GCA GCC CAT AAA
GTG TAG TAC GTT CGT CCG GTC TGT GAC GTA GAG GTT ACG TCC CCC CTT CGT CGG GTA TTT
His Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys>

          190         200         210         220         230         240
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
TGG TCT TTG CCT GAA ATG GTG AGT AAG GAA AGC GAA AGG CTG AGC ATA ACT AAA TCT GCC
ACC AGA AAC GGA CTT TAC CAC TCA TTC CTT TCG CTT TCC GAC TCG TAT TGA TTT AGA CGG
Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala>

          250         260         270         280         290         300
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
TGT GGA AGA AAT GGC AAA CAA TTC TGC AGT ACT TTA ACC TTG AAC ACA GCT CAA GCA AAC
ACA CCT TCT TTA CCG TTT GTT AAG ACG TCA TGA AAT TGG AAC TTG TGT CGA GTT CGT TTG
Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn>

          310         320         330         340         350         360
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
CAC ACT GGC TTC TAC AGC TGC AAA TAT CTA GCT GTA CCT ACT TCA AAG AAG AAG GAA ACA
GTG TGA CCG AAG ATG TCG ACG TTT ATA GAT CGA CAT GGA TGA AGT TTC TTC TTC CTT TGT
His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys Lys Lys Glu Thr>

          370         380         390         400         410         420
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
GAA TCT GCA ATC TAT ATA TTT ATT AGT GAT ACA GGT AGA CCT TTC GTA GAG ATG TAC AGT
CTT AGA CGT TAG ATA TAT AAA TAA TCA CTA TGT CCA TCT GGA AAG CAT CTC TAC ATG TCA
Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser>

          430         440         450         460         470         480
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
GAA ATC CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT
CTT TAG GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA
Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val>

          490         500         510         520         530         540
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
ACG TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT
TGC AGT GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA
Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp>
```

Fig.13B.

```
        550         560         570         580         590         600
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA
CCT TTT GCG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT
Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys>

        610         620         630         640         650         660
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GAA ATA GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT
CTT TAT CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA
Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr>

        670         680         690         700         710         720
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CTC ACA CAT CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA CCA CGC CCA GTC
GAG TGT GTA GCT GTT TGG TTA TGT TAG TAT CTA CAG GTT TAT TCG TGT GGT GCG GGT CAG
Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val>

        730         740         750         760         770         780
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAA TTA CTT AGA GGC CAT ACT CTT GTC CTC AAT TGT ACT GCT ACC ACT CCC TTG AAC ACG
TTT AAT GAA TCT CCG GTA TGA GAA CAG GAG TTA ACA TGA CGA TGG TGA GGG AAC TTG TGC
Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr>

        790         800         810         820         830         840
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AGA GTT CAA ATG ACC TGG AGT TAC CCT GAT GAA ATT GAC CAA AGC AAT TCC CAT GCC AAC
TCT CAA GTT TAC TGG ACC TCA ATG GGA CTA CTT TAA CTG GTT TCG TTA AGG GTA CGG TTG
Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Ile Asp Gln Ser Asn Ser His Ala Asn>

        850         860         870         880         890         900
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
ATA TTC TAC AGT GTT CTT ACT ATT GAC AAA ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT
TAT AAG ATG TCA CAA GAA TGA TAA CTG TTT TAC GTC TTG TTT CTG TTT CCT GAA ATA TGA
Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr>

        910         920         930         940         950         960
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TGT CGT GTA AGG AGT GGA CCA TCA TTC AAA TCT GTT AAC ACC TCA GTG CAT ATA TAT GAT
ACA GCA CAT TCC TCA CCT GGT AGT AAG TTT AGA CAA TTG TGG AGT CAC GTA TAT ATA CTA
Cys Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val His Ile Tyr Asp>

        970         980         990         1000        1010        1020
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAA GCA GGC CCG GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA
TTT CGT CCG GGC CCG CTC GGG TTT AGA ACA CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT
Lys Ala Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro>

        1030        1040        1050        1060        1070        1080
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr>
```

Fig.13C.

```
        1090        1100        1110        1120        1130        1140
    *   *   *   *   *   *   *   *   *   *   *   *
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC
GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp>

        1150        1160        1170        1180        1190        1200
    *   *   *   *   *   *   *   *   *   *   *   *
CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG
GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC TGT TTC
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys>

        1210        1220        1230        1240        1250        1260
    *   *   *   *   *   *   *   *   *   *   *   *
CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC
GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His>

        1270        1280        1290        1300        1310        1320
    *   *   *   *   *   *   *   *   *   *   *   *
CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC
GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala>

        1330        1340        1350        1360        1370        1380
    *   *   *   *   *   *   *   *   *   *   *   *
CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC
GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr>

        1390        1400        1410        1420        1430        1440
    *   *   *   *   *   *   *   *   *   *   *   *
CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA
GAC GGG GGT AGG GCC CTA CTC GAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys>

        1450        1460        1470        1480        1490        1500
    *   *   *   *   *   *   *   *   *   *   *   *
GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC
CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn>

        1510        1520        1530        1540        1550        1560
    *   *   *   *   *   *   *   *   *   *   *   *
TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC
ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu>

        1570        1580        1590        1600        1610        1620
    *   *   *   *   *   *   *   *   *   *   *   *
ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu>
```

Fig.13D.

```
        1630         1640         1650         1660         1670
     *    *    *    *    *    *    *    *    *    *
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>
```

Fig.14A.

```
          10          20          30          40          50          60
     *    *    *    *    *    *    *    *    *    *    *    *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG CGC GAC GAG TCG ACA GAC GAA GAG
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu>

          70          80          90          100         110         120
     *    *    *    *    *    *    *    *    *    *    *    *
ACA GGA TCT AGT TCC GGA GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC GAA ATT
TGT CCT AGA TCA AGG CCT CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG GGG CTT TAA
Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile>

          130         140         150         160         170         180
     *    *    *    *    *    *    *    *    *    *    *    *
ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA CCT AAC ATC
TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA TGC AGT GGA TTG TAG
Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile>

          190         200         210         220         230         240
     *    *    *    *    *    *    *    *    *    *    *    *
ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA CGC ATA ATC
TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA CCT TTT GCG TAT TAG
Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile>

          250         260         270         280         290         300
     *    *    *    *    *    *    *    *    *    *    *    *
TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG
ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT CCC GAA GAC
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu>

          310         320         330         340         350         360
     *    *    *    *    *    *    *    *    *    *    *    *
ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA CAT CGA CAA
TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT GTA GCT GTT
Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln>

          370         380         390         400         410         420
     *    *    *    *    *    *    *    *    *    *    *    *
ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA CCA CGC CCA GTC AAA TTA CTT AGA GGC
TGG TTA TGT TAG TAT CTA CAG GTT TAT TCG TGT GGT GCG GGT CAG TTT AAT GAA TCT CCG
Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val Lys Leu Leu Arg Gly>

          430         440         450         460         470         480
     *    *    *    *    *    *    *    *    *    *    *    *
CAT ACT CTT GTC CTC AAT TGT ACT GCT ACC ACT CCC TTG AAC ACG AGA GTT CAA ATG ACC
GTA TGA GAA CAG GAG TTA ACA TGA CGA TGG TGA GGG AAC TTG TGC TCT CAA GTT TAC TGG
His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr>

          490         500         510         520         530         540
     *    *    *    *    *    *    *    *    *    *    *    *
TGG AGT TAC CCT GAT GAA ATT GAC CAA AGC AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT
ACC TCA ATG GGA CTA CTT TAA CTG GTT TCG TTA AGG GTA CGG TTG TAT AAG ATG TCA CAA
Trp Ser Tyr Pro Asp Glu Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val>
```

Fig.14B.

```
          550         560         570         580         590         600
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
CTT ACT ATT GAC AAA ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT TGT CGT GTA AGG AGT
GAA TGA TAA CTG TTT TAC GTC TTG TTT CTG TTT CCT GAA ATA TGA ACA GCA CAT TCC TCA
Leu Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser>

          610         620         630         640         650         660
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
GGA CCA TCA TTC AAA TCT GTT AAC ACC TCA GTG CAT ATA TAT GAT AAA GCA GGC CCG GGC
CCT GGT AGT AAG TTT AGA CAA TTG TGG AGT CAC GTA TAT ATA CTA TTT CGT CCG GGC CCG
Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Gly Pro Gly>

          670         680         690         700         710         720
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG
CTC GGG TTT AGA ACA CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu>

          730         740         750         760         770         780
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG
CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg>

          790         800         810         820         830         840
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC
TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe>

          850         860         870         880         890         900
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG
TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC TGT TTC GGC GCC CTC CTC GTC
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln>

          910         920         930         940         950         960
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT
ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn>

          970         980         990         1000        1010        1020
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC
CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC TTT TGG
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr>

         1030        1040        1050        1060        1070        1080
     *    *    *    *    *    *    *    *    *    *    *    *    *    *
ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG
TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC GGG GGT AGG GCC
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg>
```

Fig.14C.

```
        1090        1100        1110        1120        1130        1140
    *     *     *     *     *     *     *     *     *     *     *     *
GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC
CTA CTC GAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser>

        1150        1160        1170        1180        1190        1200
    *     *     *     *     *     *     *     *     *     *     *     *
GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT
CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro>

        1210        1220        1230        1240        1250        1260
    *     *     *     *     *     *     *     *     *     *     *     *
CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC
GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG CAC CTG TTC TCG
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser>

        1270        1280        1290        1300        1310        1320
    *     *     *     *     *     *     *     *     *     *     *     *
AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC
TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His>

        1330        1340        1350
    *     *     *     *     *     *     *
TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>
```

Fig.15A.

```
          10          20          30          40          50          60
     *    *     *     *     *     *     *     *     *     *     *     *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG CGC GAC GAG TCG ACA GAC GAA GAG
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu>

          70          80          90          100         110         120
     *    *     *     *     *     *     *     *     *     *     *     *
ACA GGA TCT AGT TCC GGA GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC GAA ATT
TGT CCT AGA TCA AGG CCT CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG GGG CTT TAA
Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile>

          130         140         150         160         170         180
     *    *     *     *     *     *     *     *     *     *     *     *
ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA CCT AAC ATC
TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA TGC AGT GGA TTG TAG
Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile>

          190         200         210         220         230         240
     *    *     *     *     *     *     *     *     *     *     *     *
ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA CGC ATA ATC
TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA CCT TTT GCG TAT TAG
Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile>

          250         260         270         280         290         300
     *    *     *     *     *     *     *     *     *     *     *     *
TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG
ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT CCC GAA GAC
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu>

          310         320         330         340         350         360
     *    *     *     *     *     *     *     *     *     *     *     *
ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA CAT CGA CAA
TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT GTA GCT GTT
Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln>

          370         380         390         400         410         420
     *    *     *     *     *     *     *     *     *     *     *     *
ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA CCA CGC CCA GTC AAA TTA CTT AGA GGC
TGG TTA TGT TAG TAT CTA CAG GTT TAT TCG TGT GGT GCG GGT CAG TTT AAT GAA TCT CCG
Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val Lys Leu Leu Arg Gly>

          430         440         450         460         470         480
     *    *     *     *     *     *     *     *     *     *     *     *
CAT ACT CTT GTC CTC AAT TGT ACT GCT ACC ACT CCC TTG AAC ACG AGA GTT CAA ATG ACC
GTA TGA GAA CAG GAG TTA ACA TGA CGA TGG TGA GGG AAC TTG TGC TCT CAA GTT TAC TGG
His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr>

          490         500         510         520         530         540
     *    *     *     *     *     *     *     *     *     *     *     *
TGG AGT TAC CCT GAT GAA AAA AAT AAG AGA GCT TCC GTA AGG CGA CGA ATT GAC CAA AGC
ACC TCA ATG GGA CTA CTT TTT TTA TTC TCT CGA AGG CAT TCC GCT GCT TAA CTG GTT TCG
Trp Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser>
```

Fig.15B.

```
        550         560         570         580         590         600
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT CTT ACT ATT GAC AAA ATG CAG AAC AAA GAC
TTA AGG GTA CGG TTG TAT AAG ATG TCA CAA GAA TGA TAA CTG TTT TAC GTC TTG TTT CTG
Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys Asp>

        610         620         630         640         650         660
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAA GGA CTT TAT ACT TGT CGT GTA AGG AGT GGA CCA TCA TTC AAA TCT GTT AAC ACC TCA
TTT CCT GAA ATA TGA ACA GCA CAT TCC TCA CCT GGT AGT AAG TTT AGA CAA TTG TGG AGT
Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser>

        670         680         690         700         710         720
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GTG CAT ATA TAT GAT AAA GCA GGC CCG GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA
CAC GTA TAT ATA CTA TTT CGT CCG GGC CCG CTC GGG TTT AGA ACA CTG TTT TGA GTG TGT
Val His Ile Tyr Asp Lys Ala Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys Thr His Thr>

        730         740         750         760         770         780
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA
ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT
Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro>

        790         800         810         820         830         840
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC
TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp>

        850         860         870         880         890         900
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT
CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His>

        910         920         930         940         950         960
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC
TTA CGG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val>

        970         980         990         1000        1010        1020
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC
GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn>

        1030        1040        1050        1060        1070        1080
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA
TTT CGG GAG GGT CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>
```

Fig.15C.

```
        1090         1100         1110         1120         1130         1140
    *    *    *    *    *    *    *    *    *    *    *    *
CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG
GGT GTC CAC ATG TGG GAC GGG GGT AGG GCC CTA CTC GAC TGG TTC TTG GTC CAG TCG GAC
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu>

        1150         1160         1170         1180         1190         1200
    *    *    *    *    *    *    *    *    *    *    *    *
ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG
TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly>

        1210         1220         1230         1240         1250         1260
    *    *    *    *    *    *    *    *    *    *    *    *
CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC
GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe>

        1270         1280         1290         1300         1310         1320
    *    *    *    *    *    *    *    *    *    *    *    *
CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC
GAG ATG TCG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys>

        1330         1340         1350         1360         1370         1380
    *    *    *    *    *    *    *    *    *    *    *    *
TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG
AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro>

    *
GGT AAA TGA
CCA TTT ACT
Gly Lys ***>
```

25/55

Fig.16A.

```
          10          20          30          40          50          60
     *    *     *     *     *     *     *     *     *     *     *     *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG CGC GAC GAG TCG ACA GAC GAA GAG
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu>

          70          80          90          100         110         120
     *    *     *     *     *     *     *     *     *     *     *     *
ACA GGA TCT AGT TCA GGT TCA AAA TTA AAA GAT CCT GAA CTG AGT TTA AAA GGC ACC CAG
TGT CCT AGA TCA AGT CCA AGT TTT AAT TTT CTA GGA CTT GAC TCA AAT TTT CCG TGG GTC
Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln>

          130         140         150         160         170         180
     *    *     *     *     *     *     *     *     *     *     *     *
CAC ATC ATG CAA GCA GGC CAG ACA CTG CAT CTC CAA TGC AGG GGG GAA GCA GCC CAT AAA
GTG TAG TAC GTT CGT CCG GTC TGT GAC GTA GAG GTT ACG TCC CCC CTT CGT CGG GTA TTT
His Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys>

          190         200         210         220         230         240
     *    *     *     *     *     *     *     *     *     *     *     *
TGG TCT TTG CCT GAA ATG GTG AGT AAG GAA AGC GAA AGG CTG AGC ATA ACT AAA TCT GCC
ACC AGA AAC GGA CTT TAC CAC TCA TTC CTT TCG CTT TCC GAC TCG TAT TGA TTT AGA CGG
Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala>

          250         260         270         280         290         300
     *    *     *     *     *     *     *     *     *     *     *     *
TGT GGA AGA AAT GGC AAA CAA TTC TGC AGT ACT TTA ACC TTG AAC ACA GCT CAA GCA AAC
ACA CCT TCT TTA CCG TTT GTT AAG ACG TCA TGA AAT TGG AAC TTG TGT CGA GTT CGT TTG
Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn>

          310         320         330         340         350         360
     *    *     *     *     *     *     *     *     *     *     *     *
CAC ACT GGC TTC TAC AGC TGC AAA TAT CTA GCT GTA CCT ACT TCA AAG AAG AAG GAA ACA
GTG TGA CCG AAG ATG TCG ACG TTT ATA GAT CGA CAT GGA TGA AGT TTC TTC TTC CTT TGT
His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys Lys Lys Glu Thr>

          370         380         390         400         410         420
     *    *     *     *     *     *     *     *     *     *     *     *
GAA TCT GCA ATC TAT ATA TTT ATT AGT GAT ACA GGT AGA CCT TTC GTA GAG ATG TAC AGT
CTT AGA CGT TAG ATA TAT AAA TAA TCA CTA TGT CCA TCT GGA AAG CAT CTC TAC ATG TCA
Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser>

          430         440         450         460         470         480
     *    *     *     *     *     *     *     *     *     *     *     *
GAA ATC CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT
CTT TAG GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA
Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val>

          490         500         510         520         530         540
     *    *     *     *     *     *     *     *     *     *     *     *
ACG TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT
TGC AGT GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA
Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp>
```

Fig.16B.

```
        550         560         570         580         590         600
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA
CCT TTT GCG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT
Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys>

        610         620         630         640         650         660
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GAA ATA GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT
CTT TAT CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA
Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr>

        670         680         690         700         710         720
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CTC ACA CAT CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA CCA CGC CCA GTC
GAG TGT GTA GCT GTT TGG TTA TGT TAG TAT CTA CAG GTT TAT TCG TGT GGT GCG GGT CAG
Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val>

        730         740         750         760         770         780
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAA TTA CTT AGA GGC CAT ACT CTT GTC CTC AAT TGT ACT GCT ACC ACT CCC TTG AAC ACG
TTT AAT GAA TCT CCG GTA TGA GAA CAG GAG TTA ACA TGA CGA TGG TGA GGG AAC TTG TGC
Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr>

        790         800         810         820         830         840
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AGA GTT CAA ATG ACC TGG AGT TAC CCT GAT GAA AAA AAT AAG AAC GCT TCC GTA AGG CGA
TCT CAA GTT TAC TGG ACC TCA ATG GGA CTA CTT TTT TTA TTC TTG CGA AGG CAT TCC GCT
Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys Asn Lys Asn Ala Ser Val Arg Arg>

        850         860         870         880         890         900
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CGA ATT GAC CAA AGC AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT CTT ACT ATT GAC AAA
GCT TAA CTG GTT TCG TTA AGG GTA CGG TTG TAT AAG ATG TCA CAA GAA TGA TAA CTG TTT
Arg Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys>

        910         920         930         940         950         960
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT TGT CGT GTA AGG AGT GGA CCA TCA TTC AAA
TAC GTC TTG TTT CTG TTT CCT GAA ATA TGA ACA GCA CAT TCC TCA CCT GGT AGT AAG TTT
Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys>

        970         980         990         1000        1010        1020
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TCT GTT AAC ACC TCA GTG CAT ATA TAT GAT AAA GCA GGC CCG GGC GAG CCC AAA TCT TGT
AGA CAA TTG TGG AGT CAC GTA TAT ATA CTA TTT CGT CCG GGC CCG CTC GGG TTT AGA ACA
Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Gly Pro Gly Glu Pro Lys Ser Cys>

        1030        1040        1050        1060        1070        1080
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC
CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val>
```

Fig.16C.

```
        1090        1100        1110        1120        1130        1140
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA
AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr>

        1150        1160        1170        1180        1190        1200
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC
ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp>

        1210        1220        1230        1240        1250        1260
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC
CCG CAC CTC CAC GTA TTA CGG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr>

        1270        1280        1290        1300        1310        1320
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG
GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys>

        1330        1340        1350        1360        1370        1380
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys>

        1390        1400        1410        1420        1430        1440
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG
CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC GGG GGT AGG GCC CTA CTC GAC TGG TTC
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys>

        1450        1460        1470        1480        1490        1500
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG
TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu>

        1510        1520        1530        1540        1550        1560
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC
ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser>

        1570        1580        1590        1600        1610        1620
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG
CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly>
```

Fig.16D.

```
         1630         1640         1650         1660         1670         1680
     *    *    *    *    *    *    *    *    *    *    *    *
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TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser>

         1690         1700
     *    *    *    *
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GAG AGG GAC AGA GGC CCA TTT ACT
Leu Ser Leu Ser Pro Gly Lys ***>
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Fig.17.

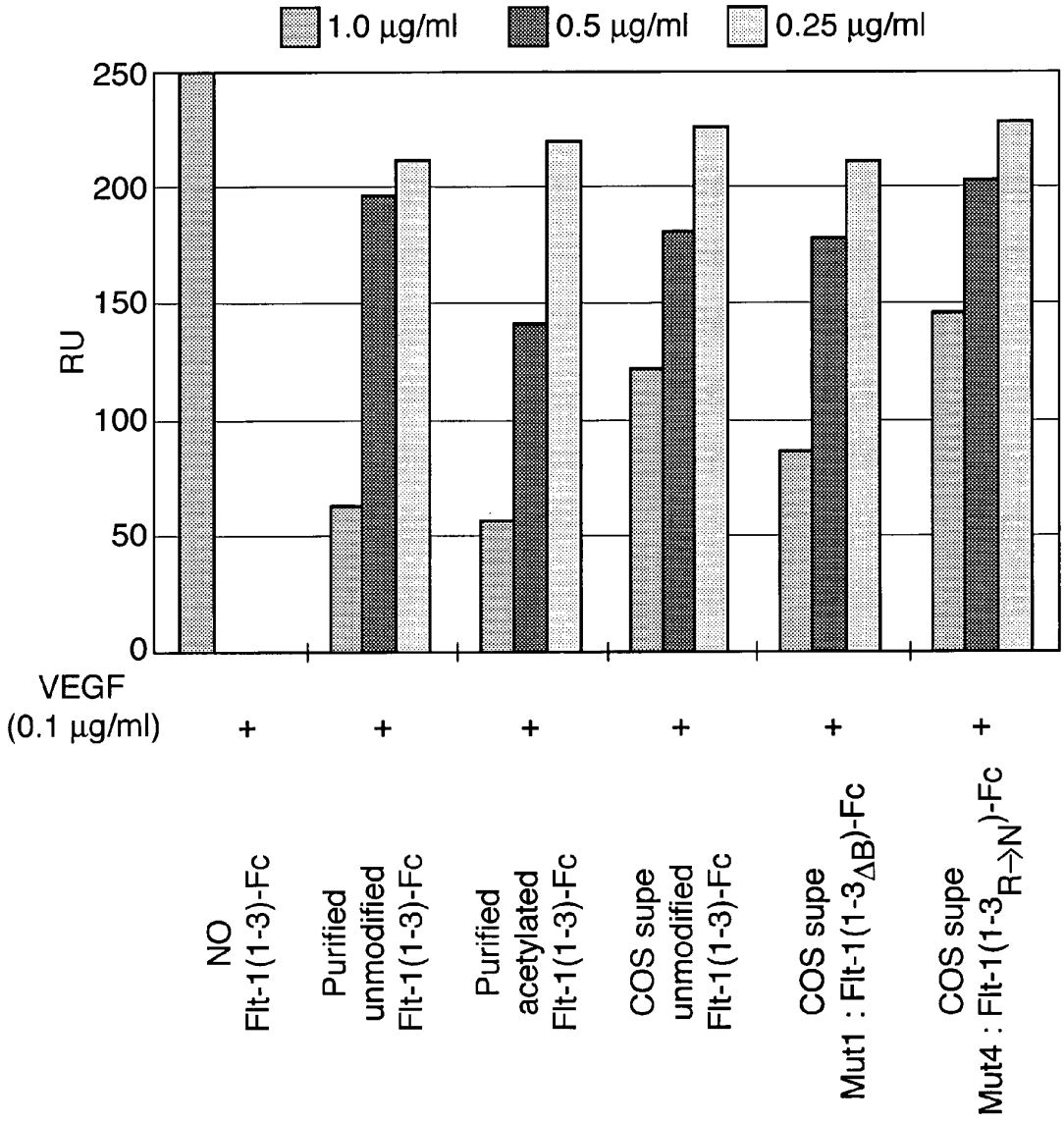

1.0 µg/ml
0.5 µg/ml
0.25 µg/ml
250
200
150
100
50
0
RU
VEGF
(0.1 µg/ml)
+
+
+
+
+
+
NO Flt-1(1-3)-Fc
Purified unmodified Flt-1(1-3)-Fc
Purified acetylated Flt-1(1-3)-Fc
COS supe unmodified Flt-1(1-3)-Fc
COS supe Mut1 : Flt-1(1-3ΔB)-Fc
COS supe Mut4 : Flt-1(1-3R→N)-Fc

Fig.18.

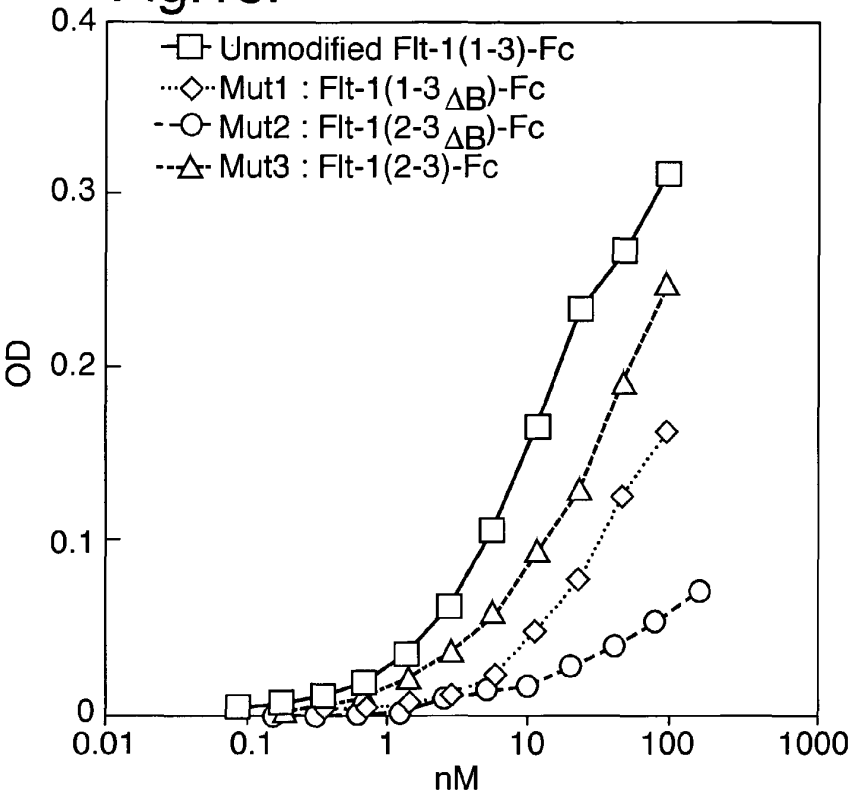

Unmodified Flt-1(1-3)-Fc
Mut1 : Flt-1(1-3 ΔB)-Fc
Mut2 : Flt-1(2-3 ΔB)-Fc
Mut3 : Flt-1(2-3)-Fc
OD
0.4
0.3
0.2
0.1
0
0.01
0.1
1
10
100
1000
nM

Fig.19.

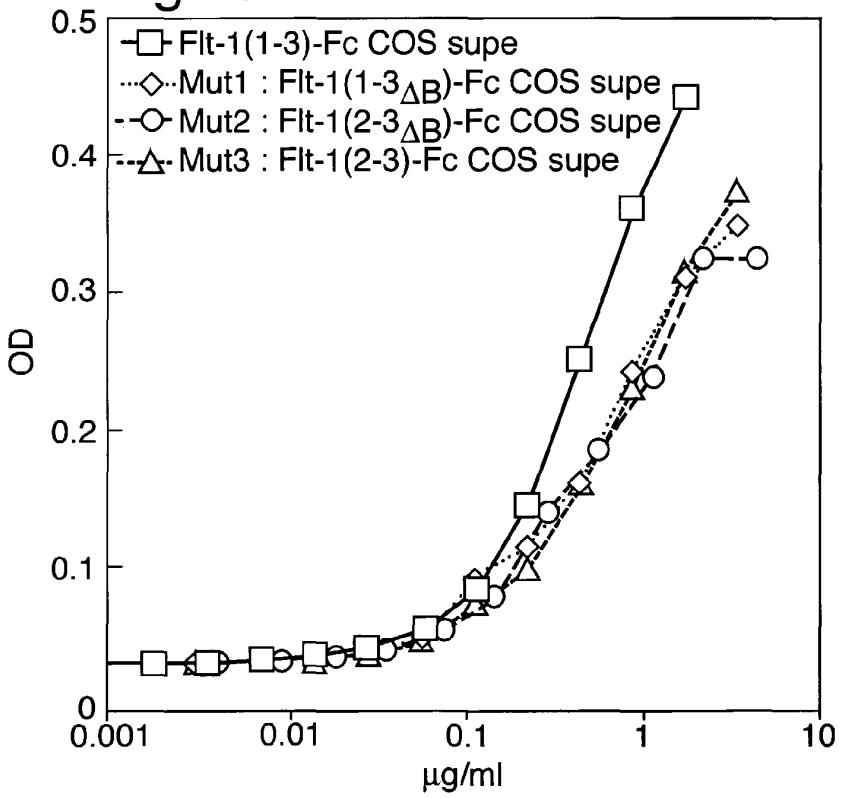

Flt-1(1-3)-Fc COS supe
Mut1 : Flt-1(1-3 ΔB)-Fc COS supe
Mut2 : Flt-1(2-3 ΔB)-Fc COS supe
Mut3 : Flt-1(2-3)-Fc COS supe
OD
0.5
0.4
0.3
0.2
0.1
0
0.001
0.01
0.1
1
10
µg/ml

Fig.20.

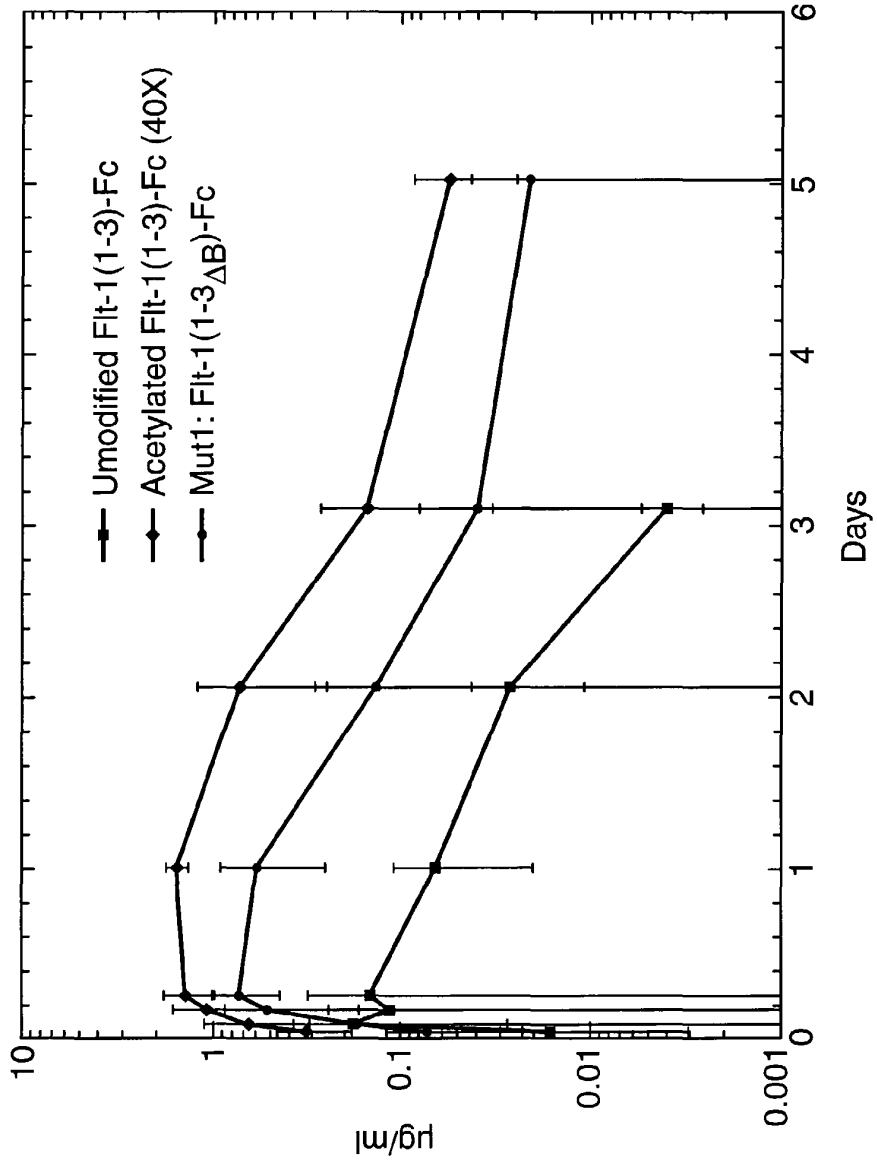

Umodified Flt-1(1-3)-Fc
Acetylated Flt-1(1-3)-Fc (40X)
Mut1: Flt-1(1-3ΔB)-Fc
μg/ml
10
1
0.1
0.01
0.001
0
1
2
3
4
5
6
Days

Fig.21A.

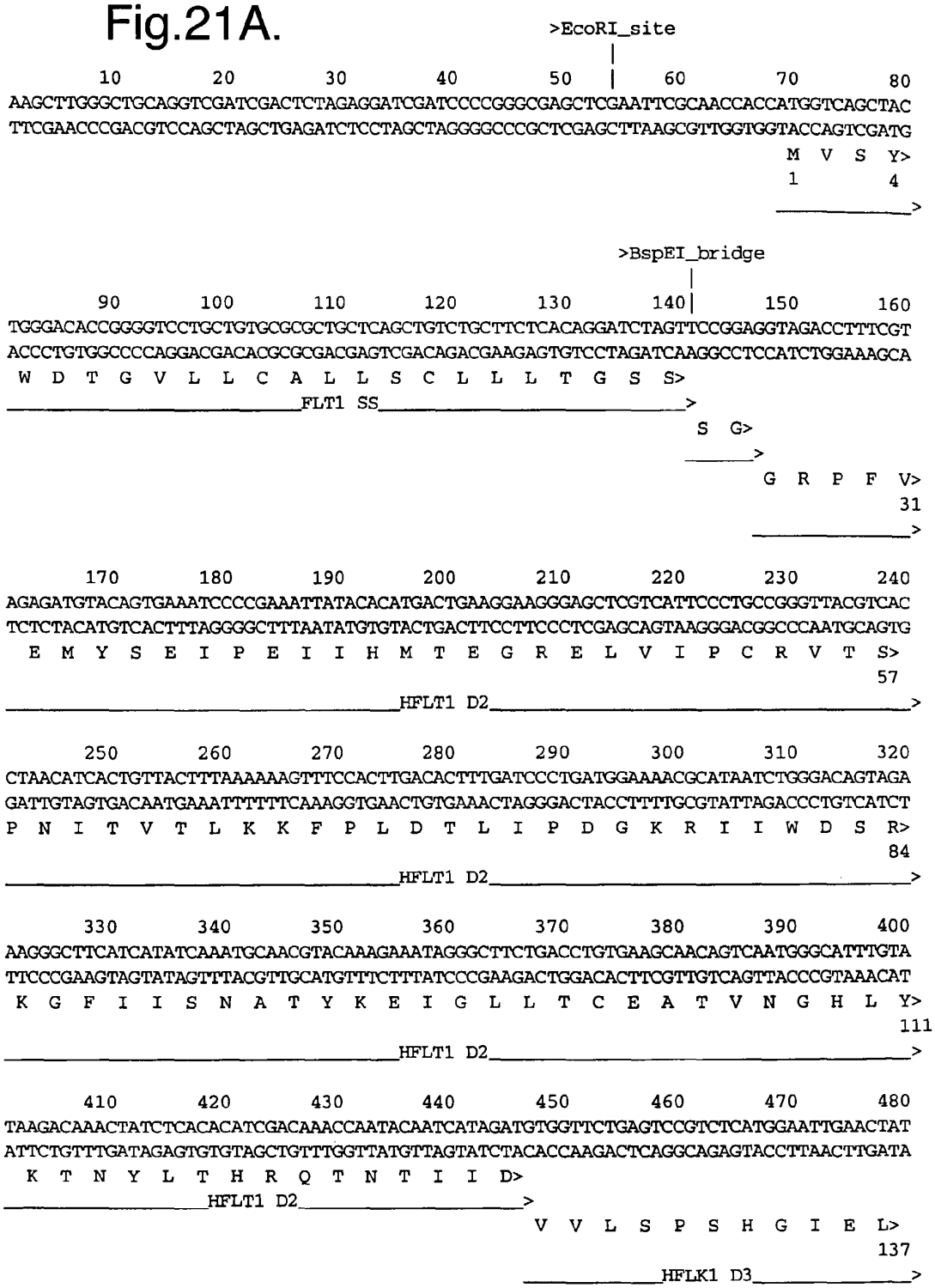

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M V S Y>
1 4
>BspEI_bridge
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W D T G V L L C A L L S C L L L T G S S>
FLT1 SS
S G>
G R P F V>
31
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57
HFLT1 D2
250 260 270 280 290 300 310 320
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84
HFLT1 D2
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111
HFLT1 D2
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HFLT1 D2
V V L S P S H G I E L>
137
HFLK1 D3

Fig.21B.

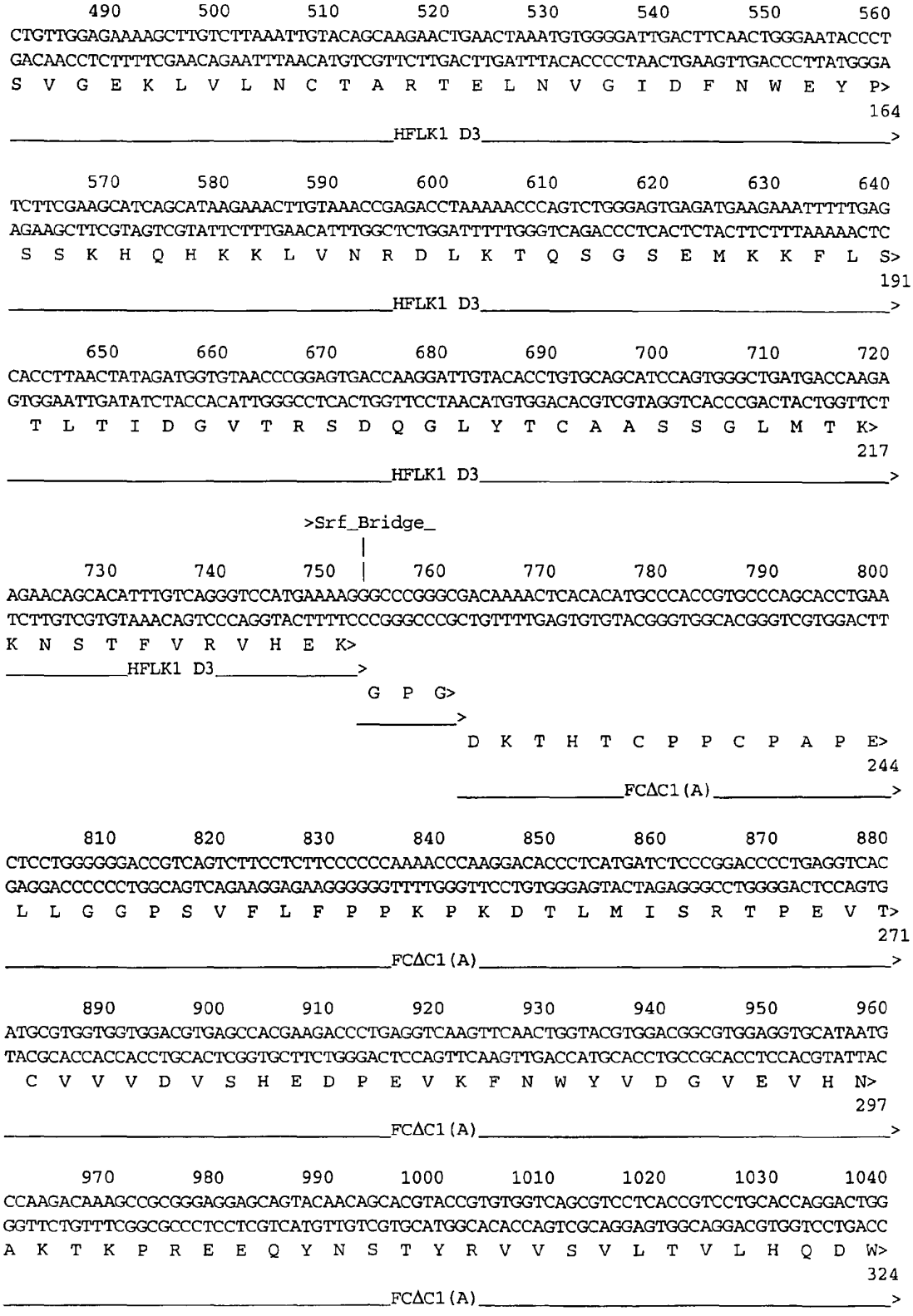

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164
HFLK1 D3
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S S K H Q H K K L V N R D L K T Q S G S E M K K F L S>
191
HFLK1 D3
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217
HFLK1 D3
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HFLK1 D3
G P G>
D K T H T C P P C P A P E>
244
FCΔC1(A)
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271
FCΔC1(A)
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297
FCΔC1(A)
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324
FCΔC1(A)

Fig.21C.

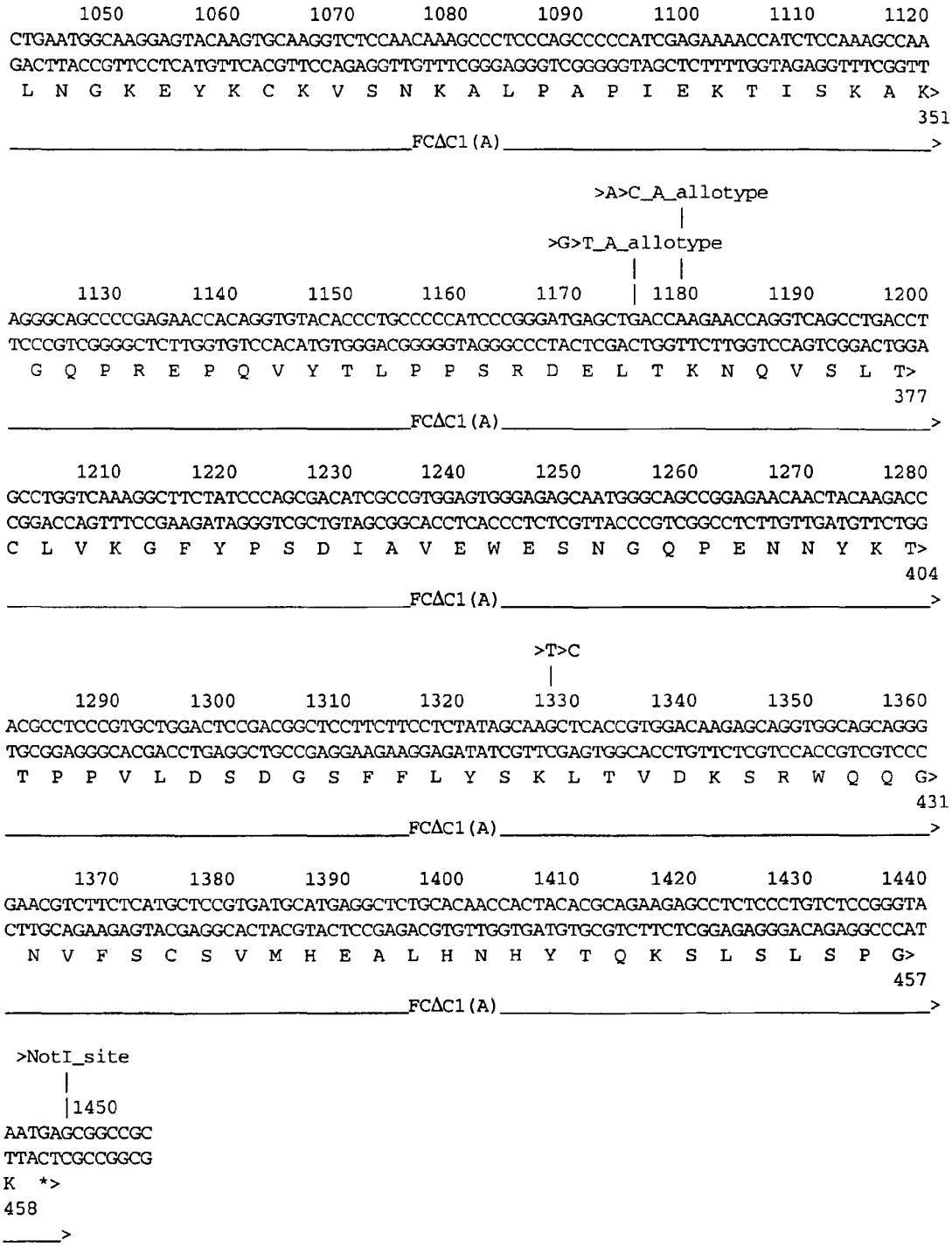

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L N G K E Y K C K V S N K A L P A P I E K T I S K A K>
351
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>G>T_A_allotype
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G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T>
377
FCΔC1(A)
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C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T>
404
FCΔC1(A)
>T>C
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T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G>
431
FCΔC1(A)
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FCΔC1(A)
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458

Fig.22A.

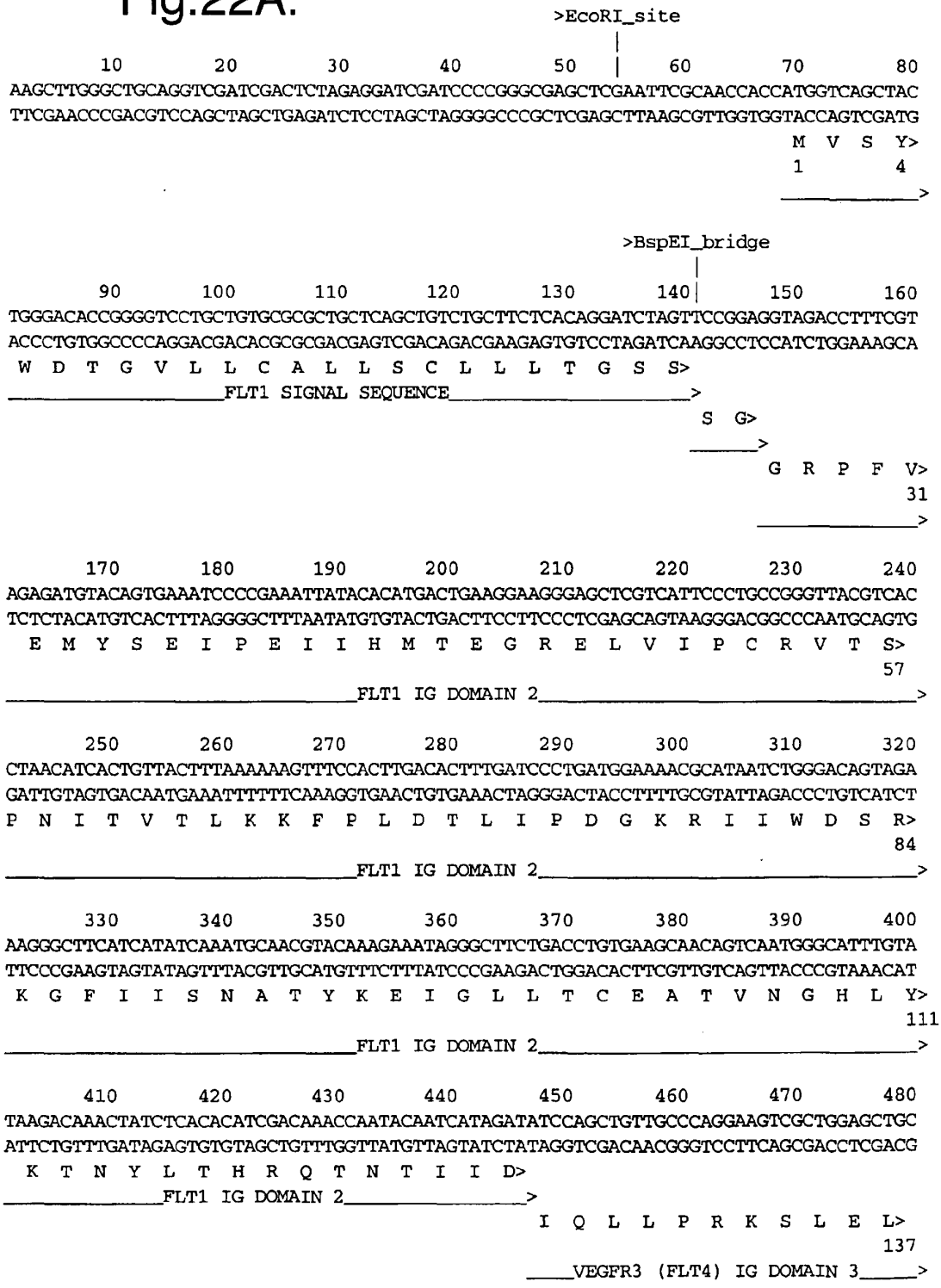

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W D T G V L L C A L L S C L L L T G S S>
FLT1 SIGNAL SEQUENCE
S G>
G R P F V>
31
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57
FLT1 IG DOMAIN 2
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84
FLT1 IG DOMAIN 2
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K G F I I S N A T Y K E I G L L T C E A T V N G H L Y>
111
FLT1 IG DOMAIN 2
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FLT1 IG DOMAIN 2
I Q L L P R K S L E L>
137
VEGFR3 (FLT4) IG DOMAIN 3

Fig.22B.

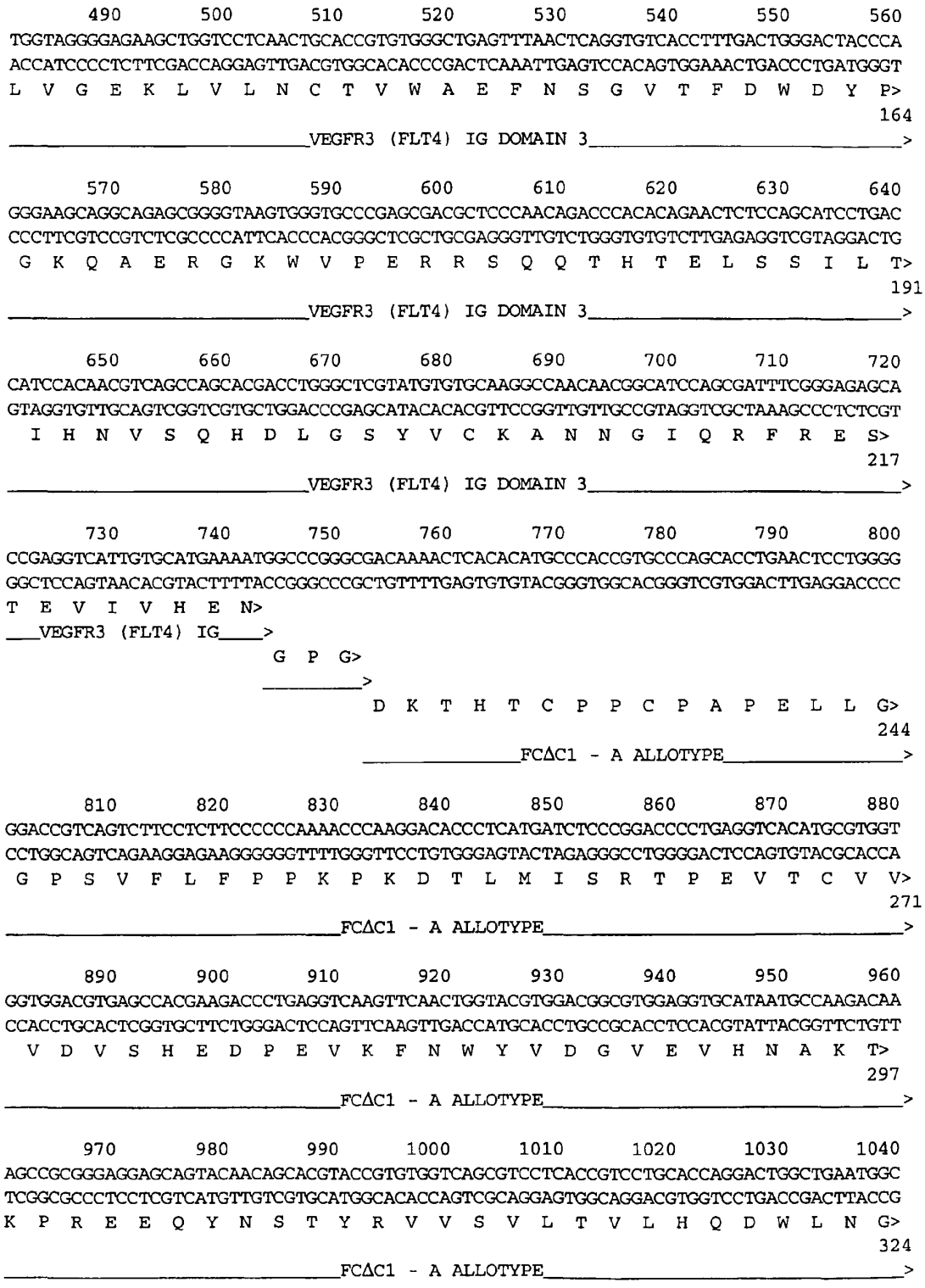

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164
VEGFR3 (FLT4) IG DOMAIN 3
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191
VEGFR3 (FLT4) IG DOMAIN 3
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217
VEGFR3 (FLT4) IG DOMAIN 3
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VEGFR3 (FLT4) IG
G P G>
D K T H T C P P C P A P E L L G>
244
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271
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297
FCΔC1 - A ALLOTYPE
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FCΔC1 - A ALLOTYPE

Fig.22C.

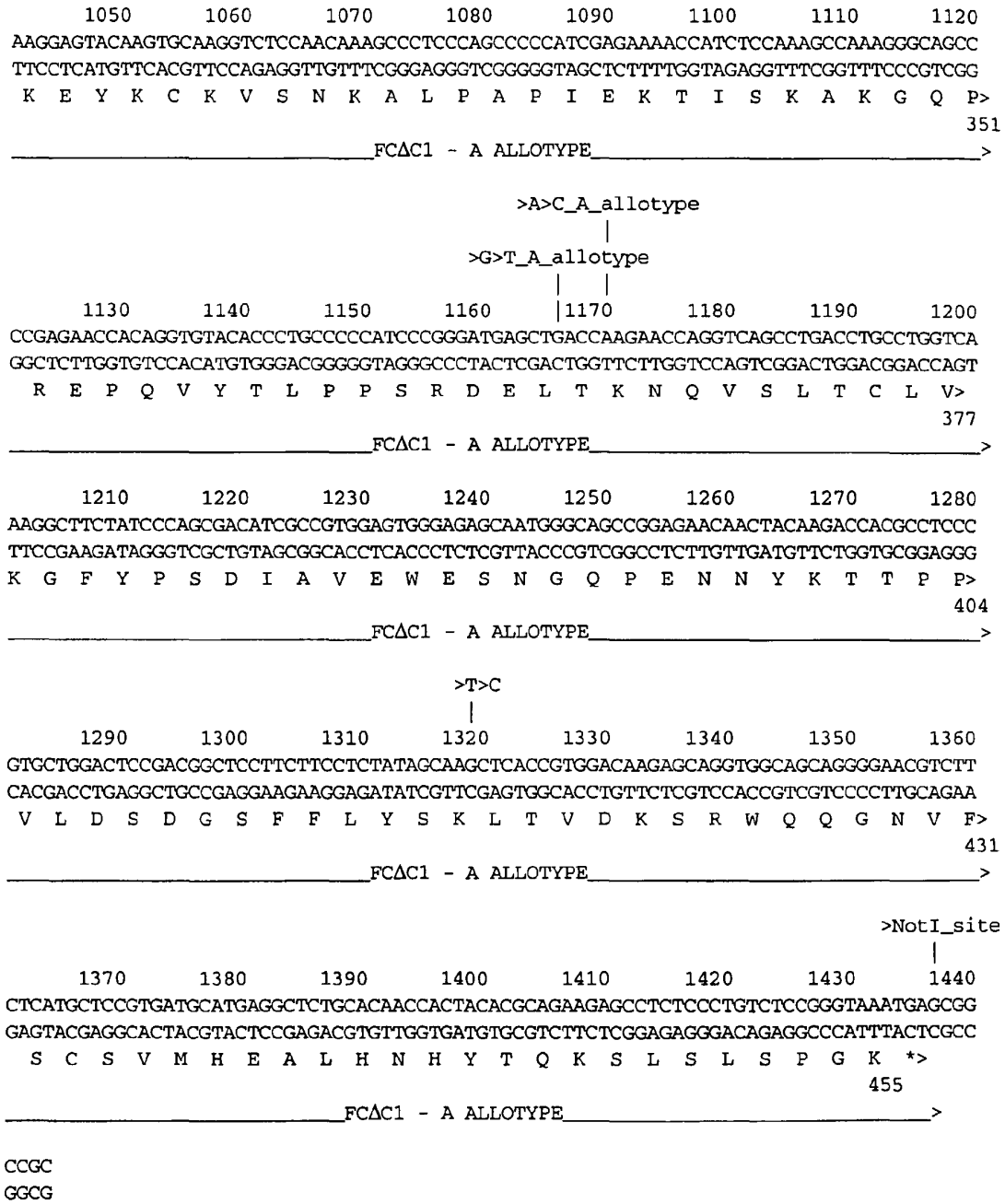

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351
FCΔC1 - A ALLOTYPE
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377
FCΔC1 - A ALLOTYPE
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404
FCΔC1 - A ALLOTYPE
>T>C
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CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGCGG
GAGTACGAGGCACTACGTACTCCGAGACGTGTTGGTGATGTGCGTCTTCTCGGAGAGGGACAGAGGCCCATTTACTCGCC
S C S V M H E A L H N H Y T Q K S L S L S P G K *>
455
FCΔC1 - A ALLOTYPE
CCGC
GGCG

Fig.23.

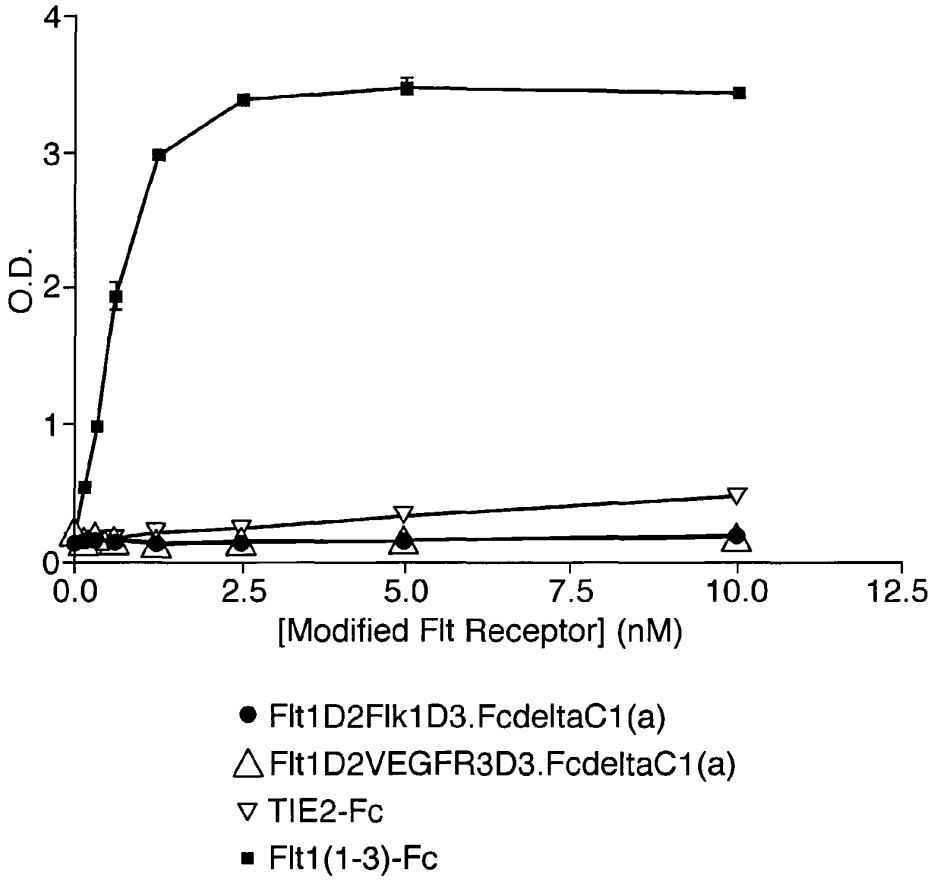

4
3
2
1
0
O.D.
0.0
2.5
5.0
7.5
10.0
12.5
[Modified Flt Receptor] (nM)
● Flt1D2Flk1D3.FcdeltaC1(a)
△ Flt1D2VEGFR3D3.FcdeltaC1(a)
▽ TIE2-Fc
■ Flt1(1-3)-Fc

Fig.24A.

```
          10          20          30          40          50          60
           *           *           *           *           *           *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG CGC GAC GAG TCG ACA GAC GAA GAG
 M   V   S   Y   W   D   T   G   V   L   L   C   A   L   L   S   C   L   L   L>
_1_____________5_________hFLT1 SIGNAL SEQUENCE________15_________________20>

          70          80          90          100         110         120
           *           *           *           *           *           *
ACA GGA TCT AGT TCC GGA AGT GAT ACC GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC
TGT CCT AGA TCA AGG CCT TCA CTA TGG CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG
 T   G   S   S   S   G>
21_hFLT1 SIGNAL SEQ_26>
                         S   D   T   G   R   P   F   V   E   M   Y   S   E   I>
                        _27_____30___hFLT1 IG DOMAIN 2_____________________40>

          130         140         150         160         170         180
           *           *           *           *           *           *
CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA
GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA TGC AGT
 P   E   I   I   H   M   T   E   G   R   E   L   V   I   P   C   R   V   T   S>
41_____________45_____________hFLT1 IG DOMAIN 2_________55_________________60>

          190         200         210         220         230         240
           *           *           *           *           *           *
CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA
GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA CCT TTT
 P   N   I   T   V   T   L   K   K   F   P   L   D   T   L   I   P   D   G   K>
61_____________65_____________hFLT1 IG DOMAIN 2_________75_________________80>

          250         260         270         280         290         300
           *           *           *           *           *           *
CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA
GCG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT
 R   I   I   W   D   S   R   K   G   F   I   I   S   N   A   T   Y   K   E   I>
81_____________85_____________hFLT1 IG DOMAIN 2_________95_________________100>

          310         320         330         340         350         360
           *           *           *           *           *           *
GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA
CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT
 G   L   L   T   C   E   A   T   V   N   G   H   L   Y   K   T   N   Y   L   T>
101____________105____________hFLT1 IG DOMAIN 2_________115________________120>

          370         380         390         400         410         420
           *           *           *           *           *           *
CAT CGA CAA ACC AAT ACA ATC ATA GAT GTG GTT CTG AGT CCG TCT CAT GGA ATT GAA CTA
GTA GCT GTT TGG TTA TGT TAG TAT CTA CAC CAA GAC TCA GGC AGA GTA CCT TAA CTT GAT
 H   R   Q   T   N   T   I   I   D>
121_____hFLT1 IG DOMAIN 2_____129_>
                                    V   V   L   S   P   S   H   G   I   E   L>
                                   130_____hFLK1 IG DOMAIN 3_____________140>
```

Fig.24B.

```
          430         440         450         460         470         480
            *           *           *           *           *           *
TCT GTT GGA GAA AAG CTT GTC TTA AAT TGT ACA GCA AGA ACT GAA CTA AAT GTG GGG ATT
AGA CAA CCT CTT TTC GAA CAG AAT TTA ACA TGT CGT TCT TGA CTT GAT TTA CAC CCC TAA
 S   V   G   E   K   L   V   L   N   C   T   A   R   T   E   L   N   V   G   I>
141_____________145_____________hFLK1 IG DOMAIN 3_____155_____________160>

          490         500         510         520         530         540
            *           *           *           *           *           *
GAC TTC AAC TGG GAA TAC CCT TCT TCG AAG CAT CAG CAT AAG AAA CTT GTA AAC CGA GAC
CTG AAG TTG ACC CTT ATG GGA AGA AGC TTC GTA GTC GTA TTC TTT GAA CAT TTG GCT CTG
 D   F   N   W   E   Y   P   S   S   K   H   Q   H   K   K   L   V   N   R   D>
161_____________165_____________hFLK1 IG DOMAIN 3_____175_____________180>

          550         560         570         580         590         600
            *           *           *           *           *           *
CTA AAA ACC CAG TCT GGG AGT GAG ATG AAG AAA TTT TTG AGC ACC TTA ACT ATA GAT GGT
GAT TTT TGG GTC AGA CCC TCA CTC TAC TTC TTT AAA AAC TCG TGG AAT TGA TAT CTA CCA
 L   K   T   Q   S   G   S   E   M   K   K   F   L   S   T   L   T   I   D   G>
181_____________185_____________hFLK1 IG DOMAIN 3_____195_____________200>

          610         620         630         640         650         660
            *           *           *           *           *           *
GTA ACC CGG AGT GAC CAA GGA TTG TAC ACC TGT GCA GCA TCC AGT GGG CTG ATG ACC AAG
CAT TGG GCC TCA CTG GTT CCT AAC ATG TGG ACA CGT CGT AGG TCA CCC GAC TAC TGG TTC
 V   T   R   S   D   Q   G   L   Y   T   C   A   A   S   S   G   L   M   T   K>
201_____________205_____________hFLK1 IG DOMAIN 3_____215_____________220>

          670         680         690         700         710         720
            *           *           *           *           *           *
AAG AAC AGC ACA TTT GTC AGG GTC CAT GAA AAG GAC AAA ACT CAC ACA TGC CCA CCG TGC
TTC TTG TCG TGT AAA CAG TCC CAG GTA CTT TTC CTG TTT TGA GTG TGT ACG GGT GGC ACG
 K   N   S   T   F   V   R   V   H   E   K>
221_____________hFLK1 IG DOMAIN 3_____________231>
                                                D   K   T   H   T   C   P   P   C>
                                              232_____________hFCAC1 A _____________240>

          730         740         750         760         770         780
            *           *           *           *           *           *
CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC
GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG
 P   A   P   E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   D>
241_____________245_____________hFCAC1 A _____________255_____________260>

          790         800         810         820         830         840
            *           *           *           *           *           *
ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA
TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT
 T   L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H   E>
261_____________265_____________hFCAC1 A _____________275_____________280>

          850         860         870         880         890         900
            *           *           *           *           *           *
GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA
CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC TGT
 D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N   A   K   T>
281_____________285_____________hFCAC1 A _____________295_____________300>
```

Fig.24C.

```
          910         920         930         940         950         960
           *           *           *           *           *           *
AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG
TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC
 K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S   V   L   T   V   L>
301_____________305_____________hFCΔC1 A _____________315_____________320>

          970         980         990        1000        1010        1020
           *           *           *           *           *           *
CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT
 H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S   N   K   A   L   P>
321_____________325_____________hFCΔC1 A _____________335_____________340>

         1030        1040        1050        1060        1070        1080
           *           *           *           *           *           *
GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC
CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG
 A   P   I   E   K   T   I   S   K   A   K   G   Q   P   R   E   P   Q   V   Y>
341_____________345_____________hFCΔC1 A _____________355_____________360>

         1090        1100        1110        1120        1130        1140
           *           *           *           *           *           *
ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC
TGG GAC GGG GGT AGG GCC CTA CTC GAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG
 T   L   P   P   S   R   D   E   L   T   K   N   Q   V   S   L   T   C   L   V>
361_____________365_____________hFCΔC1 A _____________375_____________380>

         1150        1160        1170        1180        1190        1200
           *           *           *           *           *           *
AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC
TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG
 K   G   F   Y   P   S   D   I   A   V   E   W   E   S   N   G   Q   P   E   N>
381_____________385_____________hFCΔC1 A _____________395_____________400>

         1210        1220        1230        1240        1250        1260
           *           *           *           *           *           *
AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG
TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC
 N   Y   K   T   T   P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K>
401_____________405_____________hFCΔC1 A _____________415_____________420>

         1270        1280        1290        1300        1310        1320
           *           *           *           *           *           *
CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT
GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA
 L   T   V   D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H>
421_____________425_____________hFCΔC1 A _____________435_____________440>

         1330        1340        1350        1360        1370
           *           *           *           *           *
GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT
 E   A   L   H   N   H   Y   T   Q   K   S   L   S   L   S   P   G   K   *>
441_____________445_____________hFCΔC1 A _____________455_____458____>
```

Fig.25A.

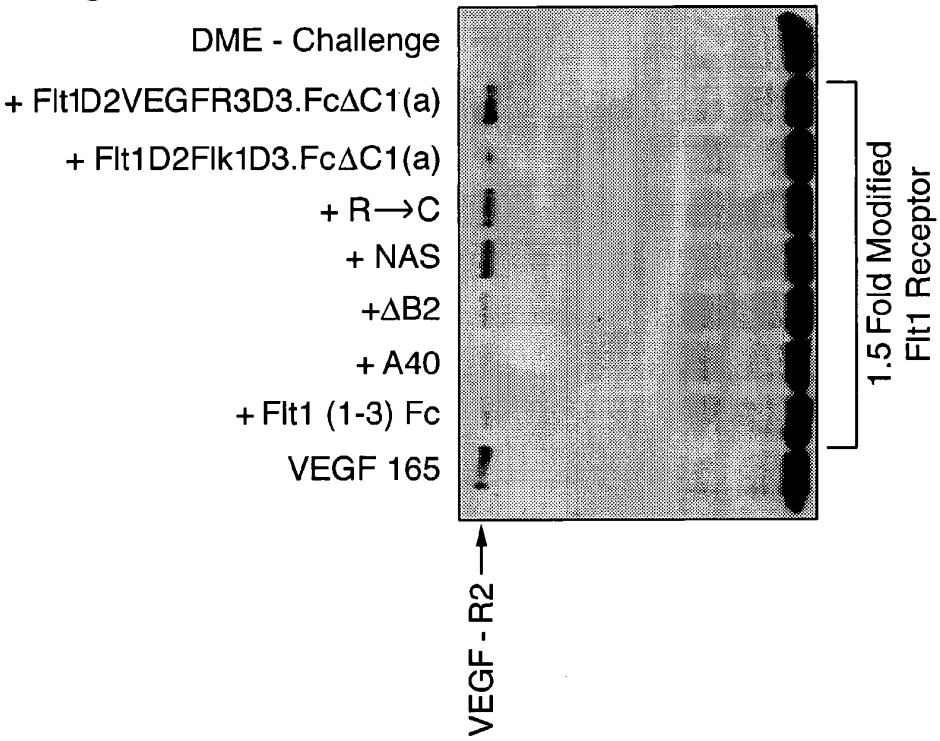


Fig.25B.

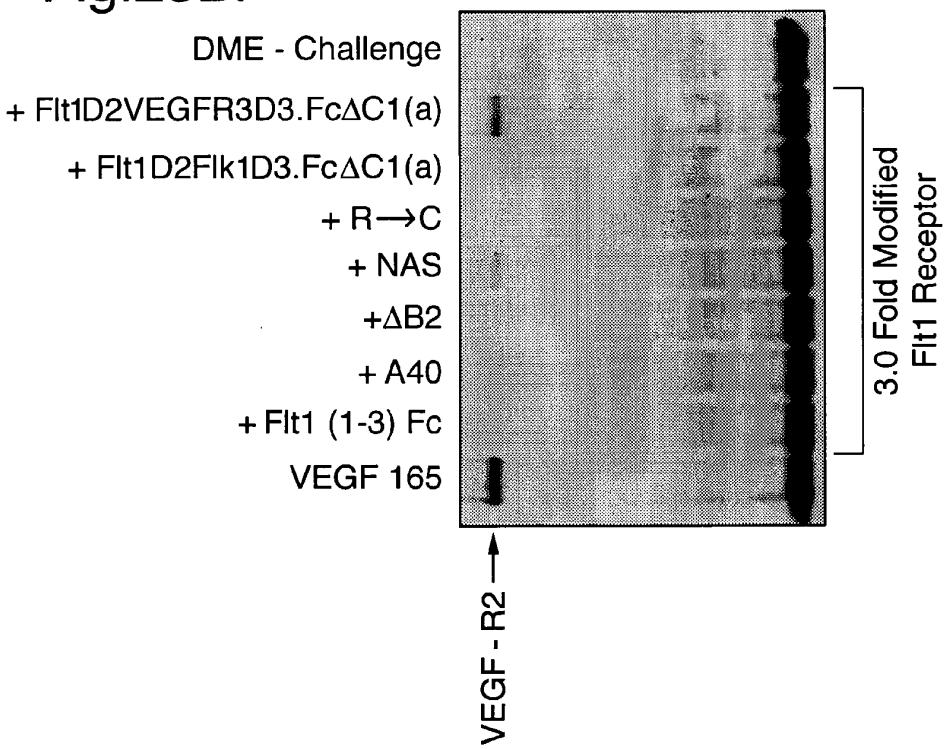


Fig.25C.

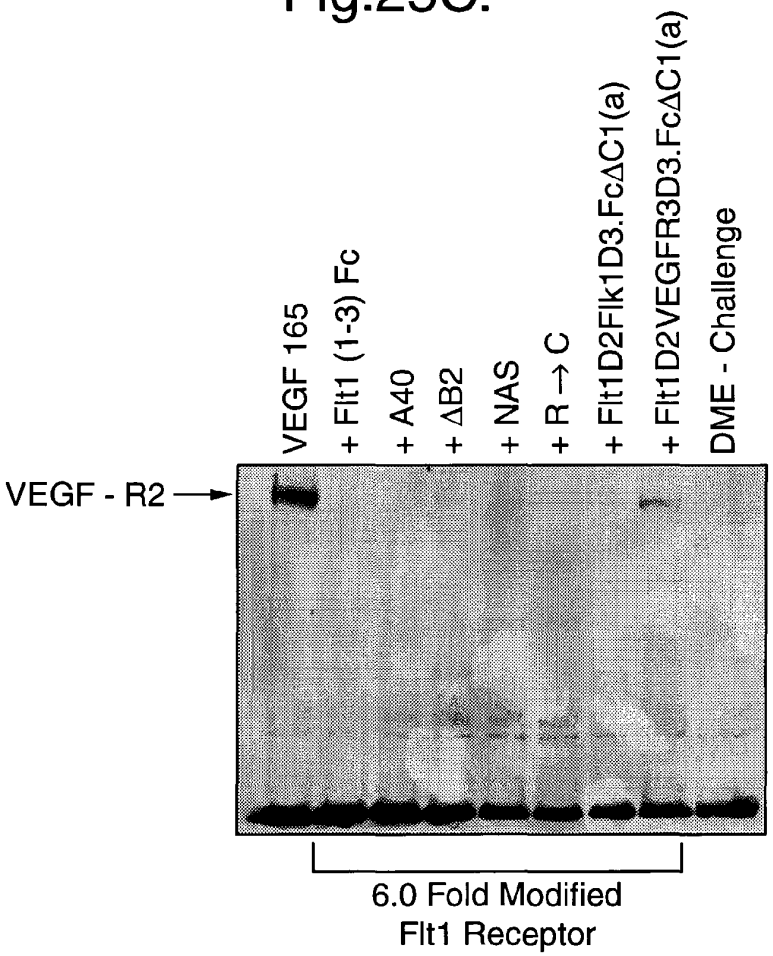

VEGF 165
+ Flt1 (1-3) Fc
+ A40
+ ΔB2
+ NAS
+ R → C
+ Flt1D2Flk1D3.FcΔC1(a)
+ Flt1D2VEGFR3D3.FcΔC1(a)
DME - Challenge
VEGF - R2
6.0 Fold Modified
Flt1 Receptor

Fig.26A.

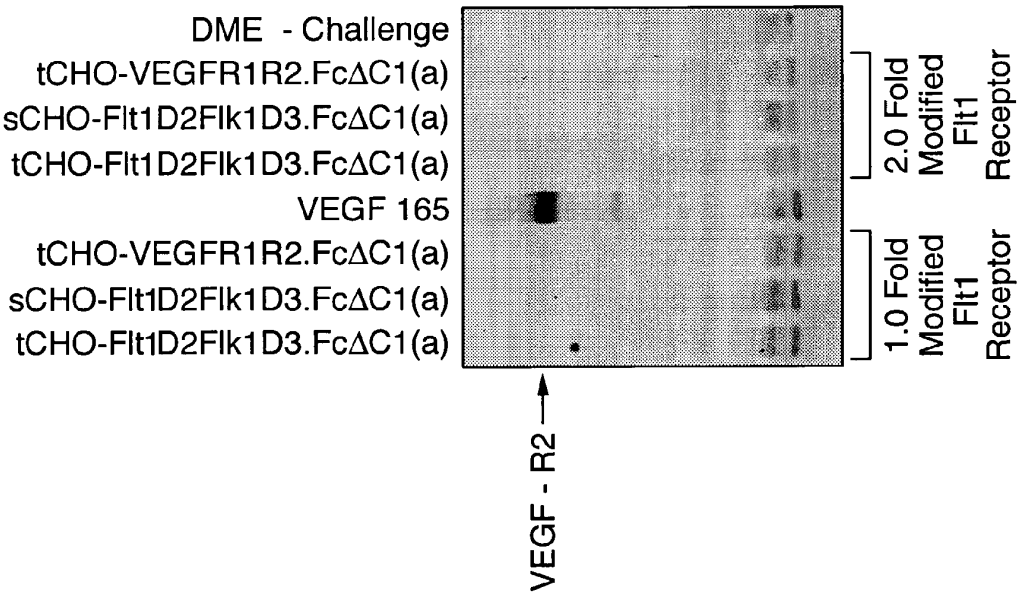


Fig.26B.

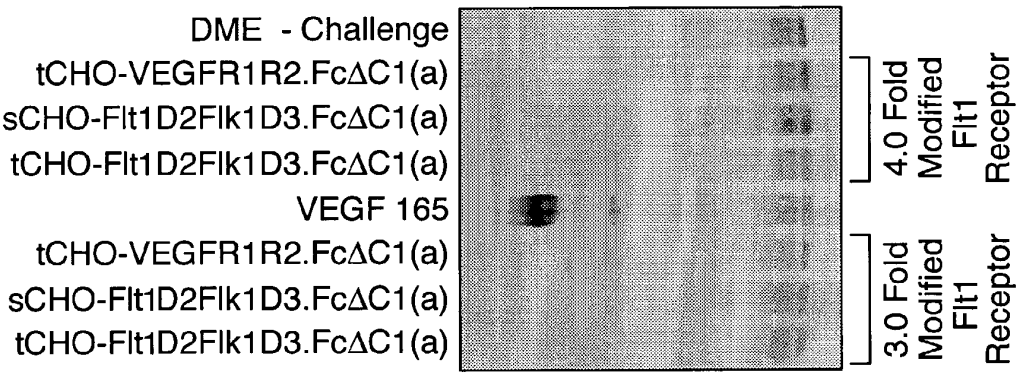


Fig.27.

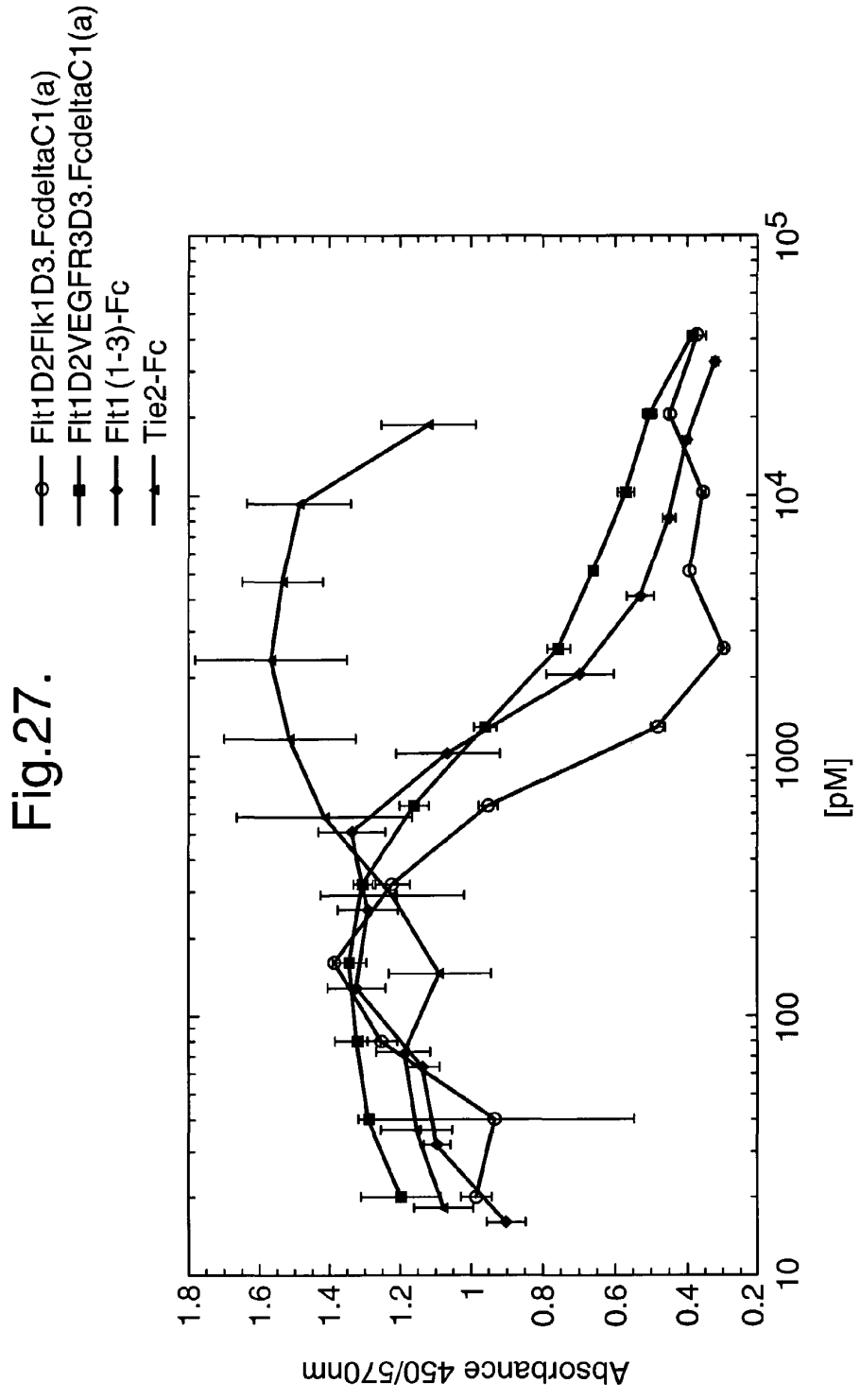

Flt1D2Flk1D3.FcdeltaC1(a)
Flt1D2VEGFR3D3.FcdeltaC1(a)
Flt1(1-3)-Fc
Tie2-Fc
Absorbance 450/570nm
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
10
100
1000
10^4
10^5
[pM]

Fig.28.

Binding Stoichiometry of hVEGF165 to Flt1D2Flk1D3.FcΔC1(a) & VEGFR1R2-FcΔC1(a)		
hVEGF165 (nM)	VEGF/Flt1D2Flk1D3.FcΔC1(a)	VEGF/VEGFR1R2-FcΔC1(a)
1	0.93	0.98
10	0.97	0.94
50	1	0.99
Average ± StDev	0.96 ± 0.03	0.97 ± 0.02

Fig.29.

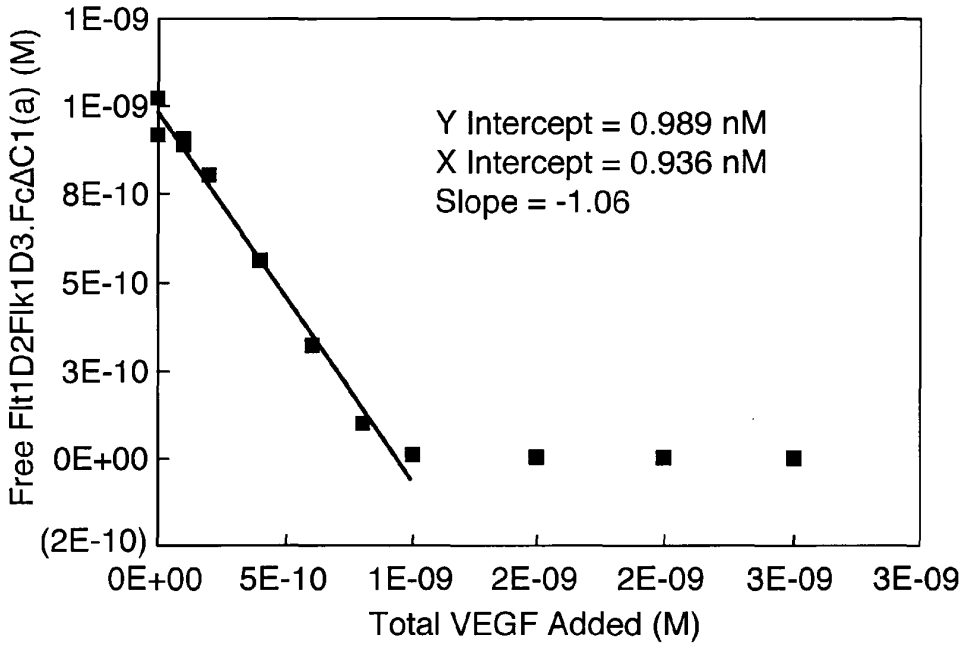

Free Flt1D2Flk1D3.FcΔC1(a) (M)
1E-09
1E-09
8E-10
5E-10
3E-10
0E+00
(2E-10)
Y Intercept = 0.989 nM
X Intercept = 0.936 nM
Slope = -1.06
0E+00
5E-10
1E-09
2E-09
2E-09
3E-09
3E-09
Total VEGF Added (M)

Fig.30.

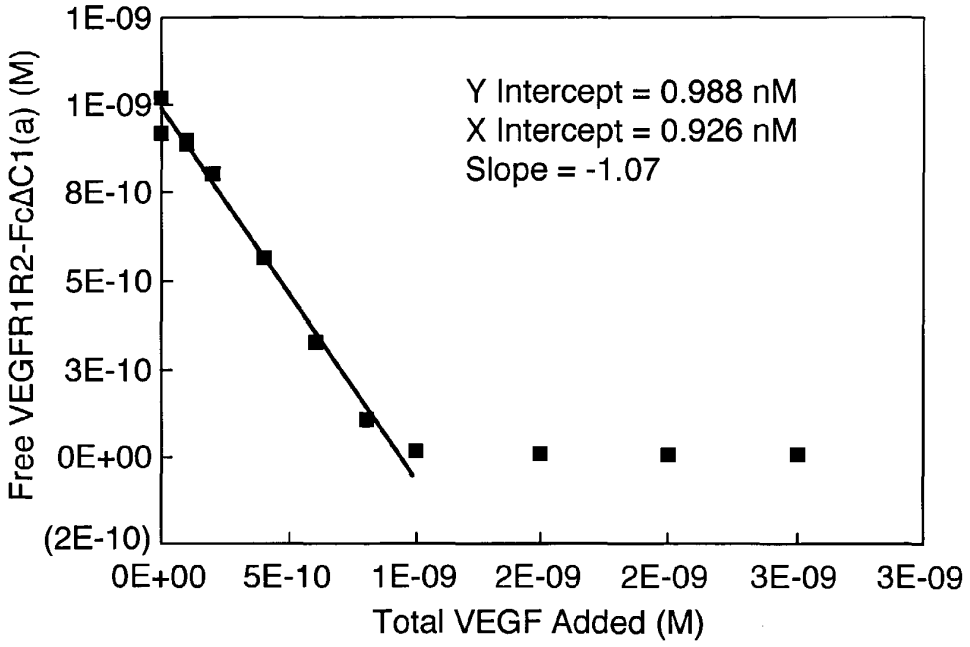

Free VEGFR1R2-FcΔC1(a) (M)
1E-09
1E-09
8E-10
5E-10
3E-10
0E+00
(2E-10)
Y Intercept = 0.988 nM
X Intercept = 0.926 nM
Slope = -1.07
0E+00
5E-10
1E-09
2E-09
2E-09
3E-09
3E-09
Total VEGF Added (M)

Fig.31.

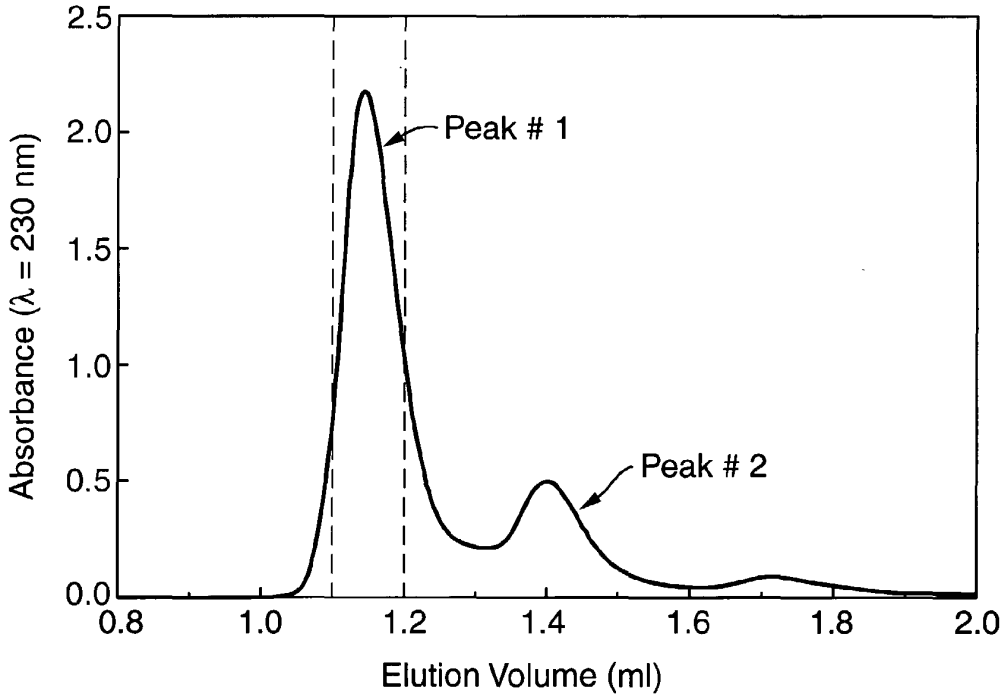

2.5
2.0
1.5
1.0
0.5
0.0
Absorbance (λ = 230 nm)
Peak # 1
Peak # 2
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Elution Volume (ml)

Fig.32.

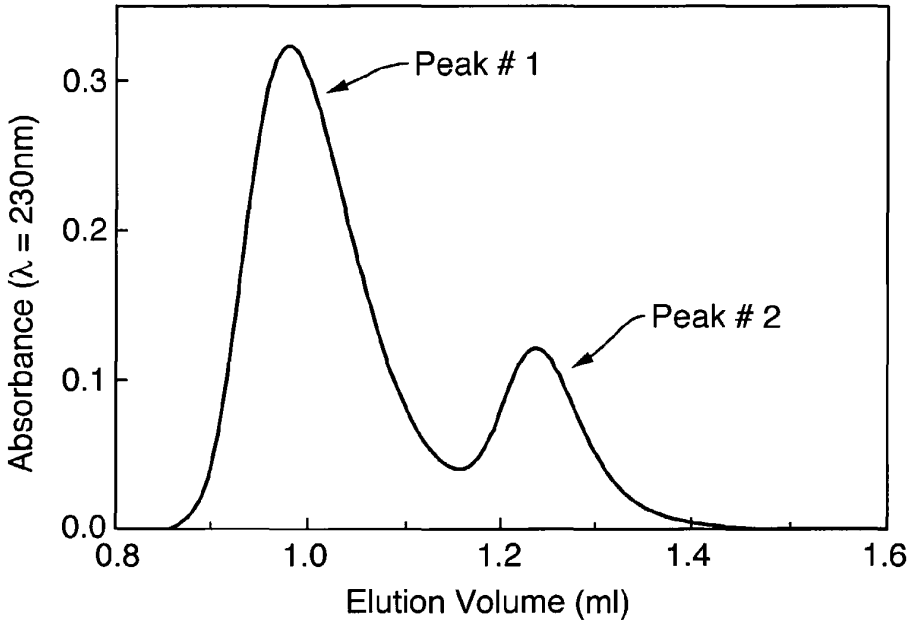

Peak # 1
Peak # 2
Absorbance (λ = 230nm)
0.0
0.1
0.2
0.3
0.8
1.0
1.2
1.4
1.6
Elution Volume (ml)

Fig.33.

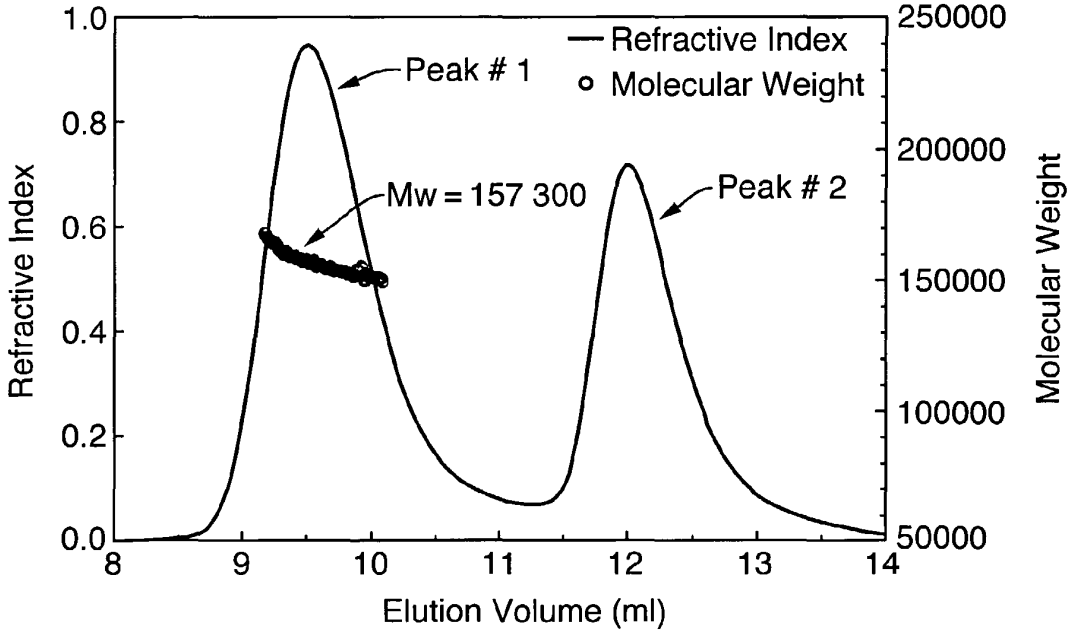

Refractive Index
Molecular Weight
Peak # 1
Mw = 157 300
Peak # 2
Refractive Index
Molecular Weight
0.0
0.2
0.4
0.6
0.8
1.0
50000
100000
150000
200000
250000
8
9
10
11
12
13
14
Elution Volume (ml)

Fig.34.

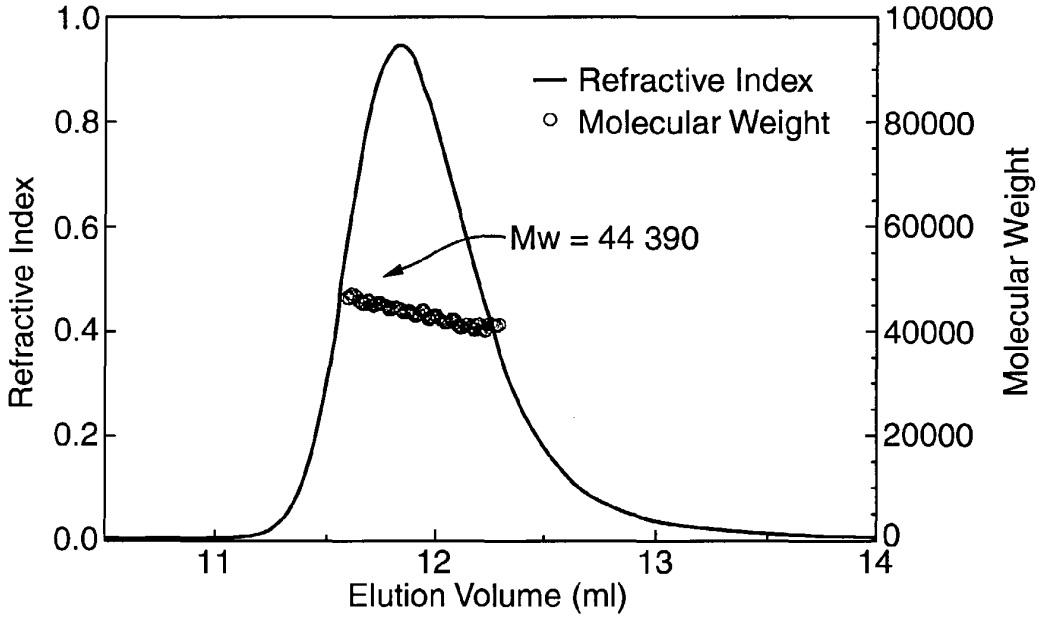

1.0
0.8
0.6
0.4
0.2
0.0
Refractive Index
100000
80000
60000
40000
20000
0
Molecular Weight
— Refractive Index
○ Molecular Weight
Mw = 44 390
11
12
13
14
Elution Volume (ml)

Fig.35.

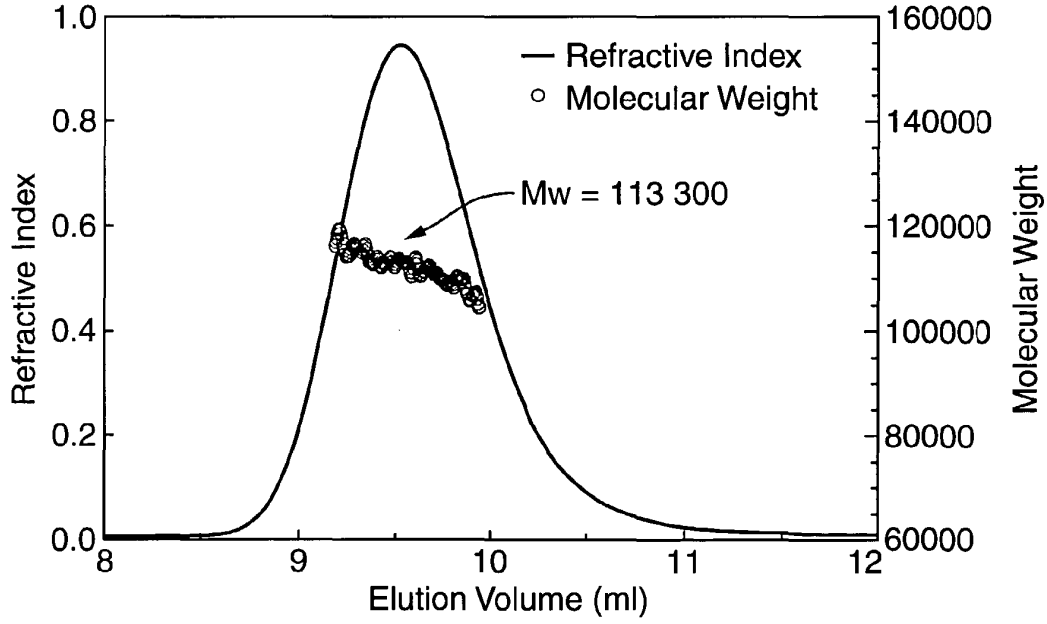

1.0
0.8
0.6
0.4
0.2
0.0
Refractive Index
160000
140000
120000
100000
80000
60000
Molecular Weight
— Refractive Index
○ Molecular Weight
Mw = 113 300
8
9
10
11
12
Elution Volume (ml)

Fig.36.

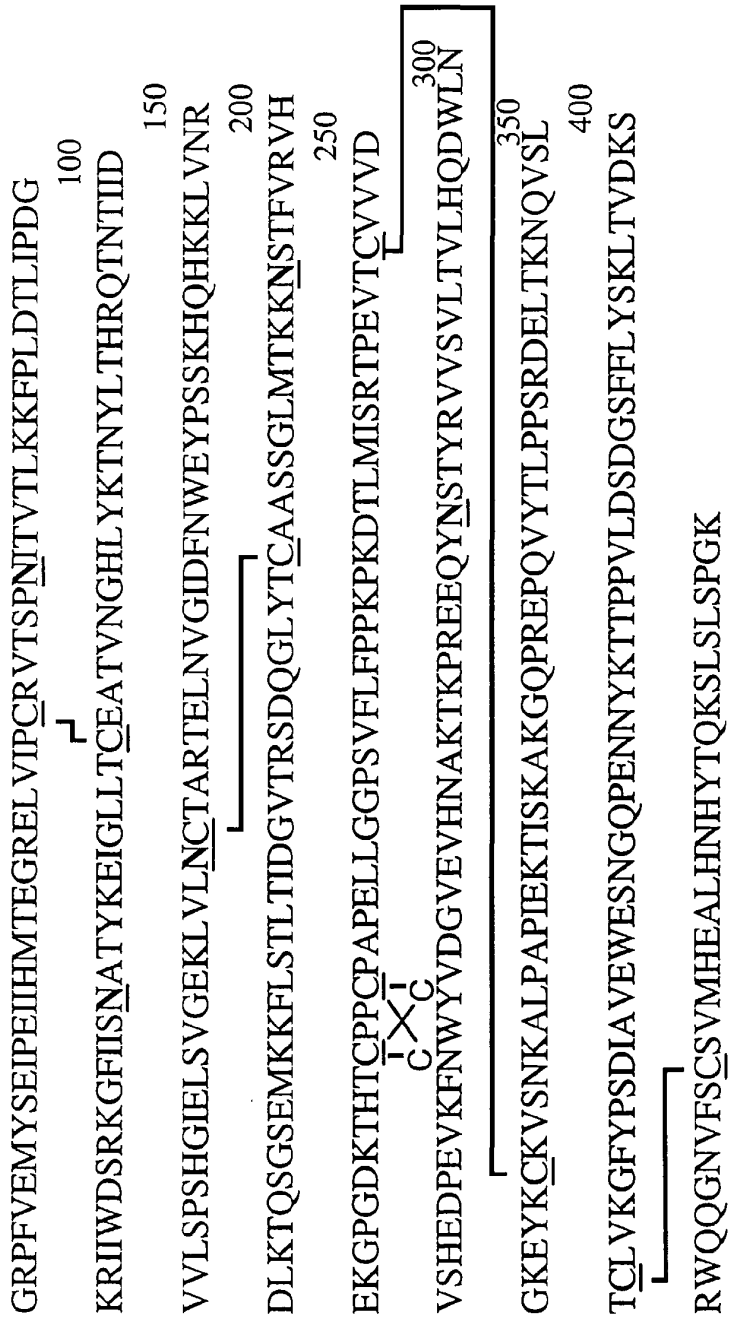

50
GRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDG
100
KRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID
150
VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNR
200
DLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVH
250
EKGPGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
C C
300
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
350
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
400
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Fig.37.

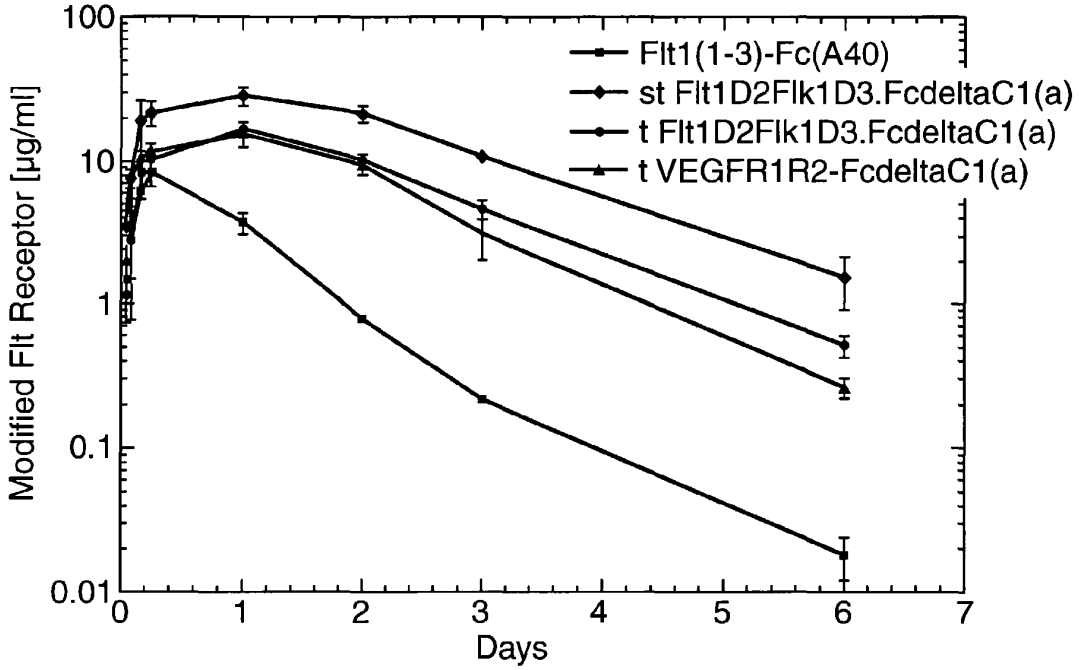

Modified Flt Receptor [µg/ml]
100
10
1
0.1
0.01
0
1
2
3
4
5
6
7
Days
Flt1(1-3)-Fc(A40)
st Flt1D2Flk1D3.FcdeltaC1(a)
t Flt1D2Flk1D3.FcdeltaC1(a)
t VEGFR1R2-FcdeltaC1(a)

Fig.38.

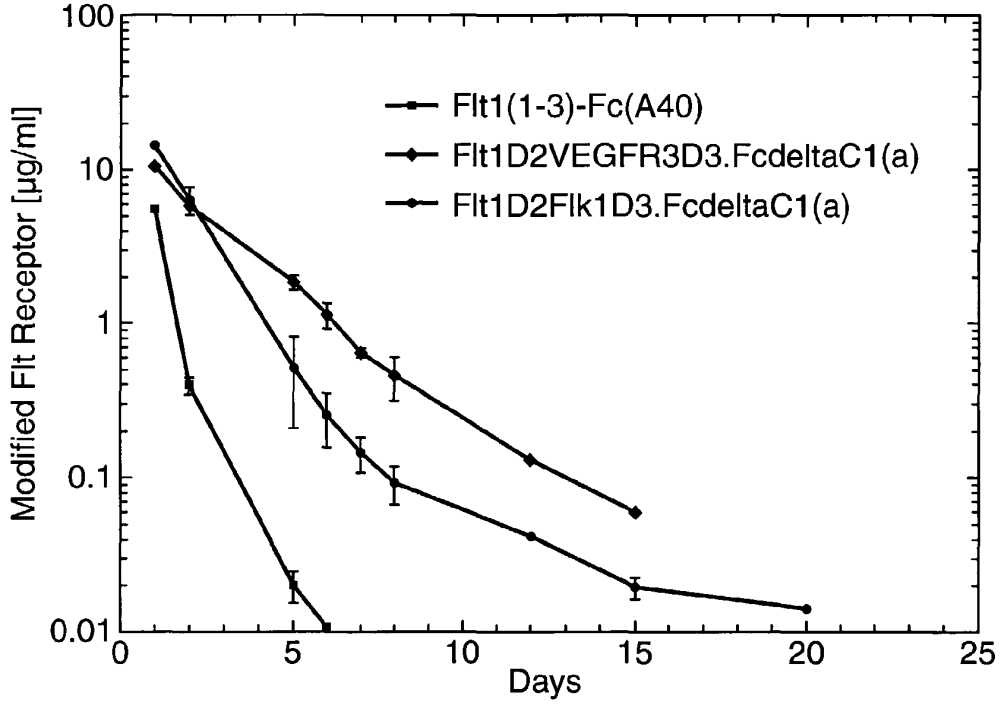

Modified Flt Receptor [µg/ml]
100
10
1
0.1
0.01
0
5
10
15
20
25
Days
Flt1(1-3)-Fc(A40)
Flt1D2VEGFR3D3.FcdeltaC1(a)
Flt1D2Flk1D3.FcdeltaC1(a)

Fig.39.

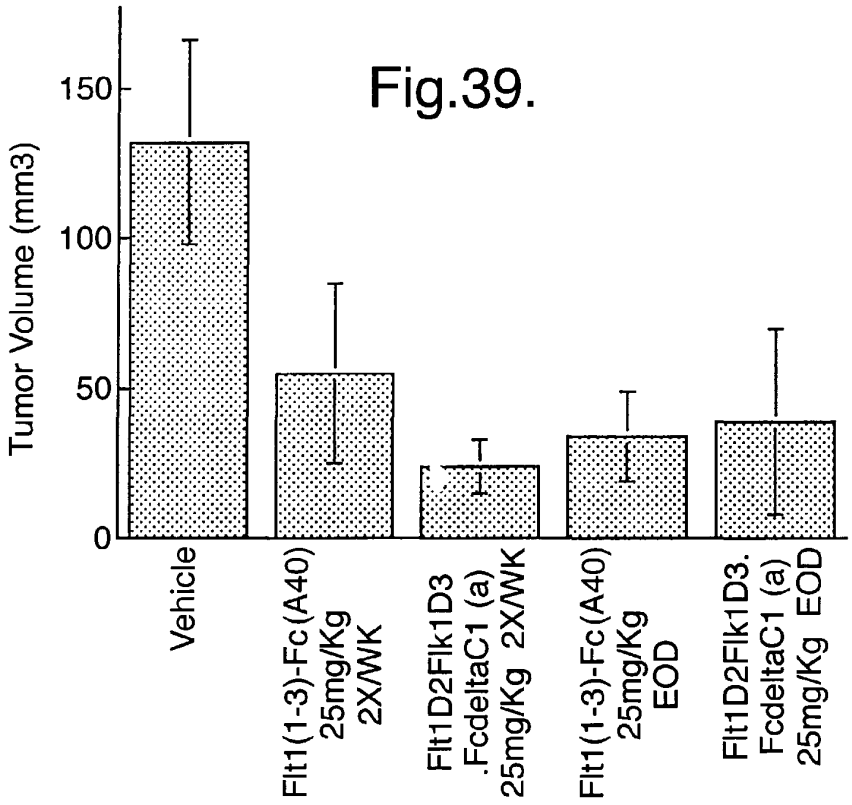

Tumor Volume (mm3)
150
100
50
0
Vehicle
Flt1(1-3)-Fc(A40) 25mg/Kg 2X/WK
Flt1D2Flk1D3 .FcdeltaC1 (a) 25mg/Kg 2X/WK
Flt1(1-3)-Fc(A40) 25mg/Kg EOD
Flt1D2Flk1D3. FcdeltaC1 (a) 25mg/Kg EOD

Fig.40.

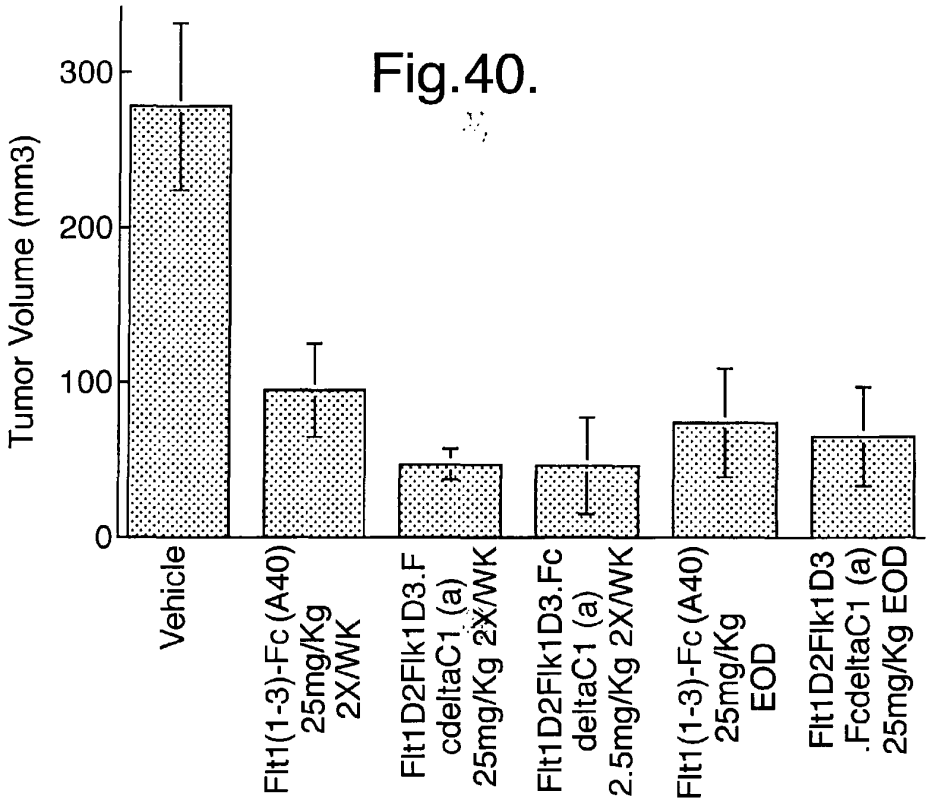

Tumor Volume (mm3)
300
200
100
0
Vehicle
Flt1(1-3)-Fc(A40) 25mg/Kg 2X/WK
Flt1D2Flk1D3.F cdeltaC1 (a) 25mg/Kg 2X/WK
Flt1D2Flk1D3.Fc deltaC1 (a) 2.5mg/Kg 2X/WK
Flt1(1-3)-Fc(A40) 25mg/Kg EOD
Flt1D2Flk1D3 .FcdeltaC1 (a) 25mg/Kg EOD

Fig.41.

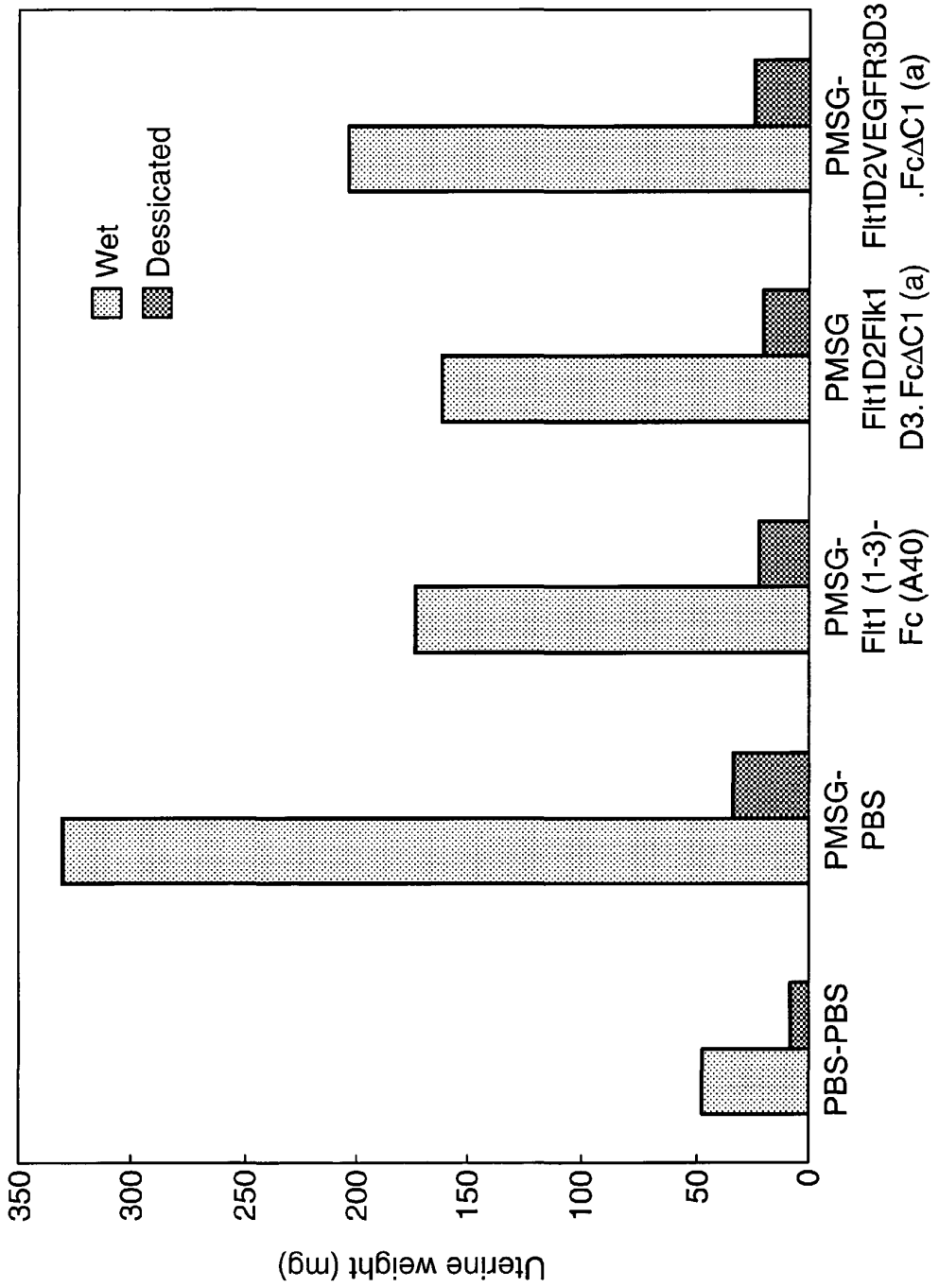

Uterine weight (mg)
350
300
250
200
150
100
50
0
Wet
Dessicated
PBS-PBS
PMSG-PBS
PMSG-Flt1 (1-3)-Fc (A40)
PMSG Flt1D2Flk1 D3. FcΔC1 (a)
PMSG-Flt1D2VEGFR3D3 .FcΔC1 (a)

Fig.42A.

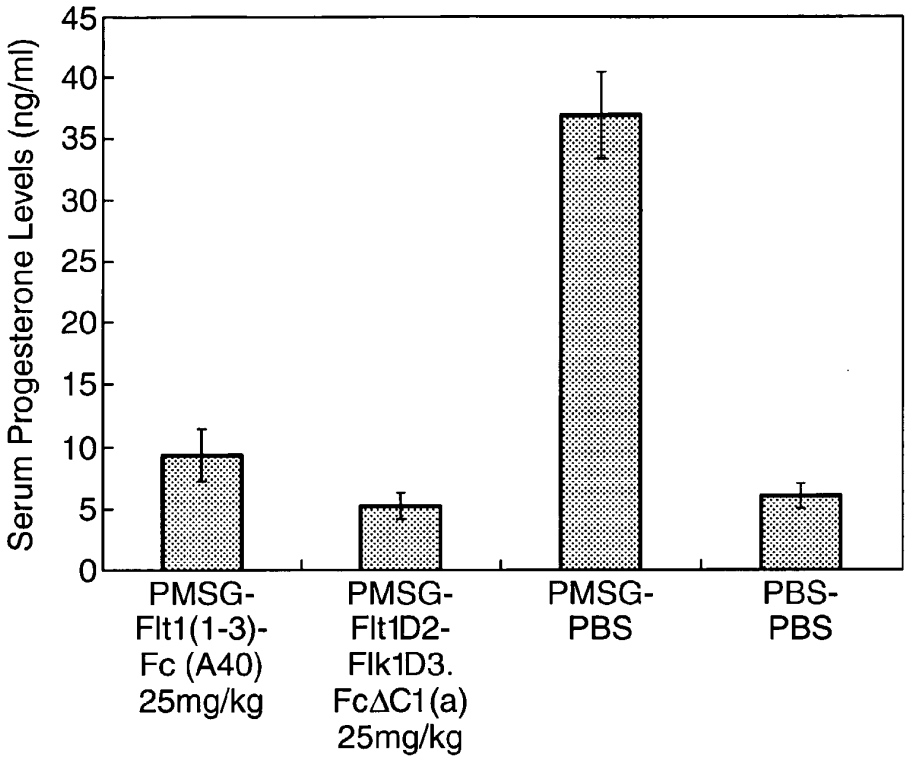
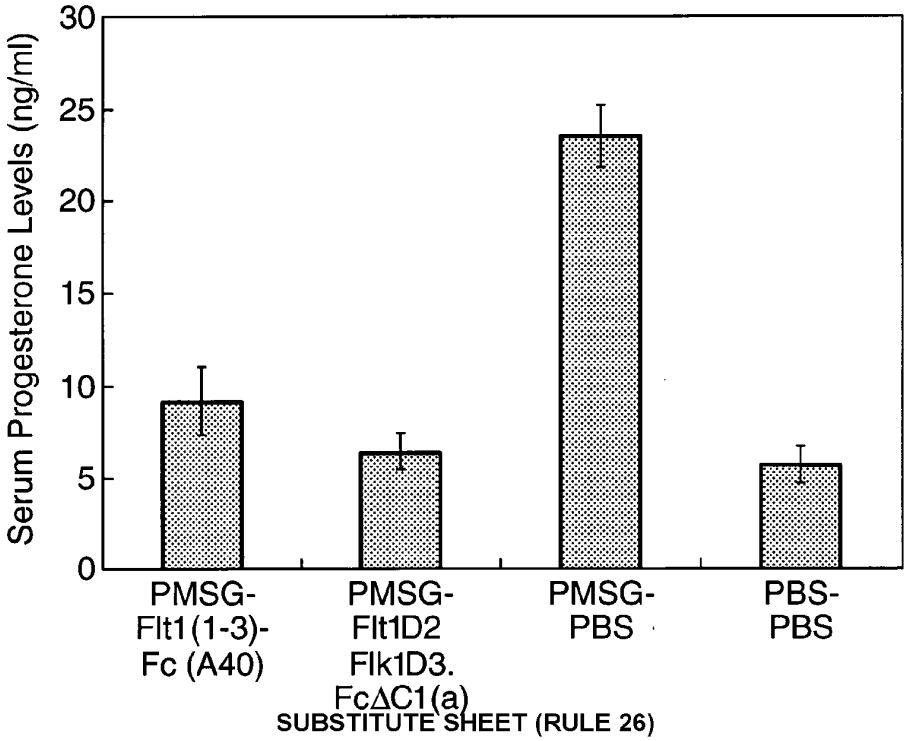


Fig.42B.



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/14142

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/62 C12N5/10 C07K14/71 A61K38/17
A61P43/00

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)
IPC 7 C12N C07K A61K A61P

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DAVIS-SMYTH T ET AL: "THE SECOND IMMUNOGLOBULIN-LIKE DOMAIN OF THE VEGF TYROSINE KINASE RECEPTOR FLT-1 DETERMINES LIGAND BINDING AND MAY INITIATE A SIGNAL TRANSDUCTION CASCADE" EMBO JOURNAL,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 15, no. 18, 16 September 1996 (1996-09-16), pages 4919-4927, XP000611912 ISSN: 0261-4189	1-21, 28-51
A	the whole document ---	22-27
A	WO 97 44453 A (GENENTECH INC ;DAVIS SMYTH TERRI LYNN (US); CHEN HELEN HSIFEI (US)) 27 November 1997 (1997-11-27) cited in the application the whole document -----	1-51

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in 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	Date of mailing of the international search report
30 August 2000	23. 11. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/14142

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 28-31, 33-40, 50, 51 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:

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 II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

see additional sheet

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. [X] 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.:

1-8, 41-48 and partly 9-40, 49-51

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

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-8, 41-48 and partly 9-40, 49-51

An isolated nuleic acid molecule encoding a fusion polypeptide capable of binding a VEGF polypeptide comprising:

(a) a nucleotide sequence encoding a VEGF receptor component operatively linked to

(b) a nucleotide sequence encoding a multimerizing component,

wherein the VEGF receptor component is the only VEGF component of the fusion polypeptide and the nucleotide sequence (a) encodes essentially an Ig domain 2 of the extracellular domain of a first VEGF receptor and an Ig domain 3 of a second VEGF receptor.

Corresponding polypeptides, vectors, recombinant host cells, compositions, therapeutic applications. Where applicable, acetylated or pegylated polypeptides.

2. Claims: partly 9-21, 28-40,49-51

An isolated nucleic acid molecule comprising a nucleotide sequence encoding a modified Flt1 receptor fusion polypeptide, wherein the coding region of the nucleic acid molecule consists of the nucleotide sequence depicted either in Fig 13A-D (seq. ID 3) or in Fig. 14A-C (Seq. ID 5).

3. Claims: partly 9-21, 28-40,49-51

Idem as subject-matter 2, but limited to the sequence depicted in Fig.15A-C (Seq. ID 7).

4. Claims: partly 9-21, 28-40,49-51

Idem as subject-matter 2, but limited to the sequence depicted in Fig.16A-D (Seq. ID 9)

5. Claims: partly 9-40,49-51

A fusion polypeptide encoded by the nucleic acid sequence set forth in Fig. 10A-D (Seq. ID 1), which has been modified by acetylation or pegylation.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/14142

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9744453 A	27-11-1997	US 6100071 A	08-08-2000
		AU 717112 B	16-03-2000
		AU 3060497 A	09-12-1997
		EP 0907733 A	14-04-1999
		JP 2000502357 T	29-02-2000
		NZ 332779 A	29-06-1999
		US 5952199 A	14-09-1999

Form PCT/ISA/210 (patent family annex) (July 1992)

Electronic Acknowledgement Receipt

EFS ID:	17164606
Application Number:	13940370
International Application Number:	
Confirmation Number:	1055
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-008CIP (725A1-US)
Receipt Date:	18-OCT-2013
Filing Date:	12-JUL-2013
Time Stamp:	13:43:52
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	Transmittal Letter	REGN-008CIP_ids_trans.pdf	35070 12f2f89001242d438c8baa9eb6eba28f7b58d129	no	3

Warnings:	
Information:	

2	Information Disclosure Statement (IDS) Form (SB08)	REGN-008CIP_ids.pdf	613767 ccfbfbc244d5883df53ec396fc1a1443069600d3	no	5
Warnings:					
Information:					
3	Non Patent Literature	Heier_2002.pdf	1983339 5a1ed4bd007fe7585823c5cb5fc7f67ba0797f60	no	10
Warnings:					
Information:					
4	Non Patent Literature	Heier_E-Abstract-2003.pdf	107636 460934f9772171eec2b2d4d7327e51f0d23e6cee	no	2
Warnings:					
Information:					
5	Non Patent Literature	NPL_cited_in_ISR.pdf	8635700 9f6d2370579c6d83ff3cb75a8655c0ea23ee5ac7	no	46
Warnings:					
Information:					
6	Non Patent Literature	Ophthalmology_2003.pdf	248276 93ee8c7eb23d88b66ff1239e31a77a0b778b446d	no	8
Warnings:					
Information:					
7	Non Patent Literature	Krzystolik_2002.pdf	5370 d5e120b8a234b7bf34b8bfcb66b4291abb1d1899	no	2
Warnings:					
Information:					
8	Non Patent Literature	Nicols_doc_guide_2003.pdf	148302 322b6d3a5da0f561c4a56bd8408b9c1c7cd0c95c	no	2
Warnings:					
Information:					
9	Foreign Reference	WO_0075319.pdf	17286091 d1580a01ef70b1a5c71f0be86656b30661f68880	no	159
Warnings:					
Information:					
		Total Files Size (in bytes):	29063551		

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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.

Electronically Filed

INFORMATION DISCLOSURE STATEMENT Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Attorney Docket	REGN-008CIP
	First Named	YANCOPOULOS, GEORGE D.
	Application Number	13/940,370
	Confirmation No.	1055
	Filing Date	July 12, 2013
	Group Art Unit	1629
	Examiner Name	
	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

Applicants submit herewith documents which may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 C.F.R. § 1.56. This submission is not intended to constitute an admission that any document referred to therein is "prior art" for this invention unless specifically designated as such. A listing of the documents is shown on enclosed Form PTO-SB/08A and a copy of each document is also enclosed.

The Examiner is requested to make the documents listed on the enclosed PTO-SB/08A of record in this application. Applicants would appreciate the Examiner initialing and returning the initialed copy of form PTO-SB/08A, indicating the document cited therein have been considered and made of record herein.

Statements

☒ **No statement**

☐ **PTA Statement under 37 CFR § 1.704(d):** Each item of information contained in the information disclosure statement filed herewith was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office and was not received by any individual designated in § 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

☐ **IDS Statement under 37 CFR § 1.97(e)(l):** 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; or

☐ **IDS Statement under 37 CFR § 1.97(e)(2):** 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 § 1.56(c) more than three months prior to the filing of the information disclosure statement.

Fees

☒ No fee is believed to be due.

☐ The appropriate fee set forth in 37 C.F.R. §1.17(p) accompanies this information disclosure statement.

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-008CIP.

Respectfully submitted,

BOZICEVIC, FIELD & FRANCIS, LLP

Date: October 18, 2013

By: /Karl Bozicevic, Reg. No.28,807/
Karl Bozicevic, Reg. No.28,807

BOZICEVIC, FIELD & FRANCIS, LLP
1900 University Avenue, Suite 200
East Palo Alto, California 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

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United States Patent and Trademark Office
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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
13/940,370	07/12/2013	George D. YANCOPOULOS	REGN-008CIP (725A1-US)

CONFIRMATION NO. 1055

96387
Regeneron
Bozicevic, Field & Francis
1900 University Ave
Suite 200
East Palo Alto, CA 94303

PUBLICATION NOTICE



Title:USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

Publication No.US-2013-0295094-A1
Publication Date:11/07/2013

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 Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

PTO/AIA/80 (07-12)
Approved for use through 11/30/2014. OMB 0651-0035
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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: 96387

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 assignments 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: 96387

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, New York 10591

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	Valerie Greene	Date	9 MAY 2013
Name	VALERIE GREENE	Telephone	914 847 1077
Title	Vice President and Associate General Counsel		

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 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.

Electronic Patent Application Fee Transmittal

Application Number:	13940370
Filing Date:	12-Jul-2013
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Filer:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIP (725A1-US)

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:				
Late Filing Fee for Oath or Declaration	1051	1	140	140
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**140**

Electronic Acknowledgement Receipt

EFS ID:	17345138
Application Number:	13940370
International Application Number:	
Confirmation Number:	1055
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-008CIP (725A1-US)
Receipt Date:	08-NOV-2013
Filing Date:	12-JUL-2013
Time Stamp:	15:01:20
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$140
RAM confirmation Number	1643
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1	Oath or Declaration filed	REGN-008CIP_declaration.pdf	173091 3a93479288a89f7da66eac0b7cd26602555bfde6	no	2
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
2	Assignee showing of ownership per 37 CFR 3.73.	REGN-008CIP_stmt_373_c_.pdf	679921 44385923bd127d6328a64f500e8ab2abae83afba	no	4
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
3	Power of Attorney	REGN_genl_poa_AIA.pdf	108269 313a44c65d8b016a8be66db3015605716a908ae0	no	1
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	30239 5d3f378028f67f63646f357f72daf629e7caf363	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	991520		

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<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.

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT International application number 13/940,370

filed on July 12, 2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: YANCOPOULOS, GEORGE D. Date (Optional): 10/20/13

Signature: X

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/AIA/01 form for each additional inventor.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. 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 1 minute 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.
10.

PTO/AIA/96 (08-12)
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STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Regeneron Pharmaceuticals, Inc.

Application No./Patent No.: 13/940,370 Filed/Issue Date: July 12, 2013

Titled: Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders

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 (chose **one** of options 1, 2, 3, 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 _____, Frame _____, 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.

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.

PTO/AIA/96 (08-12)
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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)

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.

☒ 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/	**November 8, 2013**
Signature	Date
Karl Bozicevic	**28,807**
Printed or Typed Name	Title or Registration Number

COPY

ASSIGNMENT

WHEREAS, I, **George D. YANCOPOULOS**, residing at 1519 Baptist Church Road, Yorktown Heights, NY 10598, am inventor of the invention(s) disclosed and/or claimed in the following patent application:

"USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS"

Serial No. 13/940,370; filed July 12, 2013.

WHEREAS, REGENERON PHARMACEUTICALS, INC., a corporation organized and existing under the laws of the State of New York, with offices at 777 Old Saw Mill River Road, Tarrytown, New York 10591-6707, U.S.A. (HEREINAFTER called "ASSIGNEE") is desirous of acquiring my (our) entire right, title and interest in, to, and under said application(s);

NOW, THEREFORE, in consideration of the sum of One Dollar ($1.00) to me (us) in hand paid, and other good and valuable consideration, the receipt of which is hereby acknowledged, I (WE), said ASSIGNOR(S), have sold, assigned, transferred and set over, and by these presents do hereby sell, assign, transfer and set over unto said ASSIGNEE, its successors, legal representatives, and assigns, my (our) entire right, title and interest for all countries in and to any and all inventions which are disclosed and claimed, and any and all inventions which are disclosed but not claimed, in the above-described United States application(s), and in and to said United States Application(s) and all divisions, renewals, continuations, and continuations-in-part thereof, and all Patents of the United States which may be granted thereon and all reissues and extensions thereof; and all applications for industrial property protection, including, without limitation, all applications for patents utility models, and designs which may hereafter be filed for said inventions in any country or countries foreign to the United States, together with the right to file such applications and the right to claim for the same the priority rights derived from said United States application(s) under the Patent Laws of the United States, the International Convention of 1883 and later modifications thereof, under the Patent Cooperation Treaty, under the European Patent Convention, or under any other available international agreement or under the domestic laws of the country in which any such application is filed, as may be applicable; and all forms of industrial property protection, including, without limitation, patents, utility models, inventors' certificates and designs which may be granted for said inventions in any country or countries foreign to the United States and all extensions, renewals and reissues thereof;

COPY

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AND I (WE) HEREBY authorize and request the Commissioner of Patents and Trademarks of the United States and any Official of any country or countries foreign to the United States whose duty it is to issue patents or other evidence or forms of industrial property protection on applications as aforesaid, to issue the same to the said ASSIGNEE, their successors, legal representatives and assigns, in accordance with this instrument;

AND I (WE) HEREBY covenant and agree that I (WE) have full right to convey the entire interest hereinafter assigned, and that I (WE) have not executed, and will not execute, any agreement in conflict herewith;

AND I (WE) HEREBY further covenant and agree that I (WE) will communicate to said ASSIGNEE, its successors, legal representatives and assigns, any facts known to me (us) respecting said inventions, and testify in any legal proceeding, sign all lawful papers, execute all divisional, continuing, continuation-in-part, reissue and foreign applications, make all rightful oaths, and generally do everything possible to aid said ASSIGNEE, its successors, legal representatives and assigns, to obtain and enforce proper protection for said inventions in all countries.

IN TESTIMONY WHEREOF, I hereunto set my hand and seal the day and year set opposite my signature.

Date: 8-20-15

George D. YANGOPOULOS

COPY

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
13/940,370	07/12/2013	George D. YANCOPOULOS	REGN-008CIP (725A1-US)

CONFIRMATION NO. 1055

96387
Regeneron
Bozicevic, Field & Francis
1900 University Ave
Suite 200
East Palo Alto, CA 94303

POA ACCEPTANCE LETTER

OC00000064939779

Date Mailed: 11/14/2013

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 11/08/2013.

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.

/zmoguss/

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

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/940,370	07/12/2013	George D. YANCOPOULOS	REGN-008CIP (725A1-US)	1055

96387 7590 02/26/2015

Regeneron
Bozicevic, Field & Francis
1900 University Ave
Suite 200
East Palo Alto, CA 94303

EXAMINER	
LOCKARD, JON MCCLELLAND	

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
02/26/2015	ELECTRONIC

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.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docket@bozpat.com
Zuehlke@bozpat.com

Office Action Summary	Application No. 13/940,370	Applicant(s) YANCOPOULOS, GEORGE D.	
	Examiner JON M. LOCKARD	Art Unit 1647	AIA (First Inventor to File) Status No

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

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 July 2013*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ 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.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-20* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *1-20* are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

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

Certified copies:

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)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Election/Restrictions

1. This application contains claims directed to the following patentably distinct species of VEGF antagonist: (1) anti-VEGF antibody, (2) anti-VEGF receptor antibody, and (3) VEGF receptor-based chimeric molecules. The species are independent or distinct because they have materially different structures and different functions. In addition, these species are not obvious variants of each other based on the current record.

2. Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 1-7 and 12-20 are generic.

3. There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply: The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species..

4. **Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined** even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species or grouping of patentably indistinct species**, including any claims subsequently added. An

argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

5. The election may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species or grouping of patentably indistinct species.

6. Should applicant traverse on the ground that the species, or groupings of patentably indistinct species from which election is required, are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing them to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103 or pre-AIA 35 U.S.C. 103(a) of the other species.

7. Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

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, **Joanne Hama**, can be reached on **(571) 272-2911**. 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.

/JON M LOCKARD/
Examiner, Art Unit 1647
February 22, 2015

PTO/SB/08a (01-10)
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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	13940370
	Filing Date	2013-07-12
	First Named Inventor	George D. YANCOPOULOS
	Art Unit	1647
	Examiner Name	LOCKARD, Jon McClelland
	Attorney Docket Number	REGN-008CIP

U.S.PATENTS						Remove
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	7396664		2008-07-08	Daly et al.	
If you wish to add additional U.S. Patent citation information please click the Add button.						Add

U.S.PATENT APPLICATION PUBLICATIONS						Remove
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					
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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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NON-PATENT LITERATURE DOCUMENTS			Remove
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]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13940370
	Filing Date	2013-07-12
	First Named Inventor	George D. YANCOPOULOS
	Art Unit	1647
	Examiner Name	LOCKARD, Jon McClelland
	Attorney Docket Number	REGN-008CIP

	1		☐

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

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	13940370
	Filing Date	2013-07-12
	First Named Inventor	George D. YANCOPOULOS
	Art Unit	1647
	Examiner Name	LOCKARD, Jon McClelland
	Attorney Docket Number	REGN-008CIP

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.

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

☐ A certification statement is not submitted herewith.

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	/Karl Bozicevic, Reg. No. 28,807/	Date (YYYY-MM-DD)	2015-03-17
Name/Print	Karl Bozicevic	Registration Number	28807

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.**

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 the Freedom of Information Act requires disclosure of these record s.

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 inspections 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:	21791600
Application Number:	13940370
International Application Number:	
Confirmation Number:	1055
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-008CIP (725A1-US)
Receipt Date:	17-MAR-2015
Filing Date:	12-JUL-2013
Time Stamp:	14:15:41
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	Transmittal Letter	REGN-008CIP_supp_IDS_trans_3-17-2015.pdf	46997 6d5f6064588d81a6c520262ee2cf87d28ba96a46	no	3

Warnings:	
Information:	

2	Information Disclosure Statement (IDS) Form (SB08)	REGN-008CIP_IDS.pdf	612229 f7eddcb13ec97f5b93690265008308bb45bf6740	no	4
Warnings:					
Information:					
			Total Files Size (in bytes):	659226	

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.

Electronically Filed

INFORMATION DISCLOSURE STATEMENT Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450		
	Attorney Docket	REGN-008CIP
	First Named	YANCOPOULOS, GEORGE D.
	Application Number	13/940,370
	Confirmation No.	1055
	Filing Date	July 12, 2013
	Group Art Unit	1647
	Examiner Name	LOCKARD, JON MCCLELLAND
	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

Applicants submit herewith documents which may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 C.F.R. § 1.56. This submission is not intended to constitute an admission that any document referred to therein is "prior art" for this invention unless specifically designated as such. A listing of the documents is shown on enclosed Form PTO-SB/08A and a copy of each document is also enclosed.

The Examiner is requested to make the documents listed on the enclosed PTO-SB/08A of record in this application. Applicants would appreciate the Examiner initialing and returning the initialed copy of form PTO-SB/08A, indicating the document cited therein have been considered and made of record herein.

+

Statements

☐ **No statement**

.....

☐ **PTA Statement under 37 CFR § 1.704(d):** Each item of information contained in the information disclosure statement filed herewith was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office and was not received by any individual designated in § 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

☒ **IDS Statement under 37 CFR § 1.97(e)(l):** 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; or

☐ **IDS Statement under 37 CFR § 1.97(e)(2):** 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 § 1.56(c) more than three months prior to the filing of the information disclosure statement.

Fees

☒ No fee is believed to be due.

☐ The appropriate fee set forth in 37 C.F.R. §1.17(p) accompanies this information disclosure statement.

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-008CIP.

Respectfully submitted,

BOZICEVIC, FIELD & FRANCIS LLP

Date: March 17, 2015

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic, Reg. No. 28,807

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
East Palo Alto, California 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

Electronically Filed March 30, 2015

RESPONSE TO ELECTION REQUIREMENT Address to: Mail Stop Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Docket No.	REGN-008CIP
	Application No.	13/940,370
	Confirmation No.	1055
	Filing Date	July 12, 2013
	Examiner	LOCKARD, JON MCCLELLAND
	Group Art Unit	1647

Sir:

This communication is submitted in response to the Election Requirement dated February 26, 2015. The Examiner therein required election of one of the following species:

(1) Anti-VEGF antibodies; and

(2) Anti-VEGF receptor antibodies; and

(3) VEGF receptor based chimeric molecules.

In response, applicants elect species (3) directed to VEGF receptor based chimeric molecules without traverse.

The requirement did not include a Restriction Requirement.

The claims 1-7 and 12-20 are generic to all of the species. The claims 9-11 are directed to the elected species (3).

If after conducting the search with respect to the elected species (3) the Examiner does not find the elected species the remaining claims 1-7 and 12-20 generic to all the species should be examined.

The Applicants expressly reserve the right under 35 USC §121 to file a divisional application directed to the non-elected subject matter or any subject matter disclosed in this application during the pendency of this application.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 and 1.17 which may be required by this paper, or to credit any overpayment, to Deposit Account No. 50-0815, order number REGN-008CIP.

Respectfully submitted,

BOZICEVIC, FIELD & FRANCIS LLP

Date: 30 March 2015

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic, Registration No. 28,807

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
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Facsimile: (650) 327-3231

Electronic Acknowledgement Receipt

EFS ID:	21912723
Application Number:	13940370
International Application Number:	
Confirmation Number:	1055
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-008CIP (725A1-US)
Receipt Date:	30-MAR-2015
Filing Date:	12-JUL-2013
Time Stamp:	14:33:08
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	Response to Election / Restriction Filed	REGN-008CIP_resp_rest_req_2-26-2015.pdf	41182 be00f3bb9e62cf2b25450f6bc3d05df5632613d3	no	2
Warnings:					
Information:					

Total Files Size (in bytes):	41182

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.

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 NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/940,370	07/12/2013	George D. YANCOPOULOS	REGN-008CIP (725A1-US)	1055

96387 7590 06/23/2015

Regeneron
Bozicevic, Field & Francis
1900 University Ave
Suite 200
East Palo Alto, CA 94303

EXAMINER	
LOCKARD, JON MCCLELLAND	

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
06/23/2015	ELECTRONIC

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.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docket@bozpat.com

Office Action Summary	Application No. 13/940,370	Applicant(s) YANCOPOULOS, GEORGE D.	
	Examiner JON M. LOCKARD	Art Unit 1647	AIA (First Inventor to File) Status No

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

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 March 2015*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ 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.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-20* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-20* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *12 July 2013* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

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

Certified copies:

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)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election of VEGF receptor based chimeric molecules as the species of VEGF antagonist (filed 30 March 2015) is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

The requirement is still deemed proper and is therefore made FINAL.

Status of Application, Amendments, and/or Claims

2. The present application is being examined under the pre-AIA first to invent provisions.

3. The response filed on 230 March 2015 has been entered in full. Claims 1-20 are pending and the subject of this Office Action. The claims also read upon the elected species of VEGF receptor based chimeric molecules.

4. The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

> (a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with

which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 1-9 and 12-20 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

6. The claims are drawn quite broadly to a method for treating an angiogenic eye disorder in a patient, said method comprising sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist. The claims also recite wherein the VEGF antagonist is a VEGF receptor-based chimeric molecule. To provide adequate written description and evidence of possession of a claimed genus, the specification must provide sufficient distinguishing identifying characteristics of the genus. The factors to be considered include disclosure of complete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, methods of making the claimed product, and any combination thereof. In this case, the only factor present in the claims is a desired functional property in the form of the recitation of being a VEGF antagonist, which the specification teaches means "any molecule that blocks, reduces or interferes with the normal biological activity of VEGF." However, there does not appear to be an adequate written description in the specification as filed of any essential structural features common to molecules

that have those desired properties, nor does it teach a commensurate number of the claimed species of VEGF antagonists that would be therapeutically beneficial within the claimed dosage schedule. While the specification provides adequate written description for a VEGF receptor-based chimeric molecule encoded by the nucleic acid of SEQ ID NO:1, or comprises (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130-231 of SEQ ID NO:2; and (3) a multimerization component comprising amino acids 232-457 of SEQ ID NO:2, it does not provide adequate written description for a commensurate number of the claimed species of VEGF antagonists. The distinguishing characteristics of the claimed genus are not described. The only adequately described species are the VEGF receptor-based chimeric molecules referred to *supra*. Accordingly, the specification does not provide adequate written description of the claimed genus.

7. *Vas-Cath Inc. v. Mahurkar*, 19USPQ2d 1111, clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed*." (See page 1117.) The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." (See *Vas-Cath* at page 1116).

8. With the exception of the polypeptides referred to above, the skilled artisan cannot envision the detailed chemical structure of the encompassed agonists, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. Adequate written description requires more than a mere statement

that it is part of the invention and reference to a potential method of isolating it. The product itself is required. See *Fiers v. Revel*, 25 USPQ2d 1601 at 1606 (CAFC 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016.

9. One cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481 at 1483. In *Fiddes*, claims directed to mammalian FGF's were found to be unpatentable due to lack of written description for that broad class. The specification provided only the bovine sequence.

10. Therefore, only a VEGF receptor-based chimeric molecule encoded by the nucleic acid of SEQ ID NO:1, or comprising (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130-231 of SEQ ID NO:2; and (3) a multimerization component comprising amino acids 232-457 of SEQ ID NO:2, but not the full breadth of the claims, meet the written description provision of 35 U.S.C. §112, first paragraph. Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

Double Patenting

11. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either

anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

12. A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

13. The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit http://www.uspto.gov/forms/. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

14. Claims 1-20 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-5 of U.S. Patent No. 7,303,746. Although the conflicting claims are not identical, they are not patentably distinct from each other because the claims of the ‘746 patent are drawn to methods for treating retinal neovascularization, comprising administering a fusion polypeptide which comprises the amino acid sequence of SEQ

ID NO:16, which shares 100% sequence identity to the fusion protein encoded by SEQ ID NO:1 of the instant application. While the '746 patent does not disclose the dosing schedules set forth in the instant claims, it is routine experimentation to optimize dosages and dosage schedules. The courts have determined that:

> "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller,* 220 F.2d 454, 454, 105 USPQ 223,235, (CCPA 1955).

Therefore, the claims are overlapping in scope.

15. Claims 1-20 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-6 of U.S. Patent No. 7,303,747. Although the conflicting claims are not identical as they differ in scope, they are not patentably distinct from each other because claims 1-6 of the '747 patent are drawn to methods for treating or ameliorating an eye disorder, including choroidal neovascularization, vascular leak, or retinal edema, comprising administering a fusion polypeptide capable of binding endothelial growth factor (VEGF) to a patient in need thereof, wherein the fusion polypeptide comprises the amino acid sequence of SEQ ID NO:6, which shares 100% sequence identity to the fusion protein encoded by SEQ ID NO:1 of the instant application. While the '747 patent does not disclose the dosing schedules set forth in the instant claims, it is routine experimentation to optimize dosages and dosage schedules. The courts have determined that:

> "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller,* 220 F.2d 454, 454, 105 USPQ 223,235, (CCPA 1955).

Therefore, the claims are overlapping in scope.

16. Claims 1-20 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-11 of U.S. Patent No. 7,306,799. Although the conflicting claims are not identical, they are not patentably distinct from each other because claims 1-6 of the '799 patent are drawn to a method for treating an eye disorder, including age-related macular degeneration and diabetic retinopathy, comprising administering a fusion polypeptide having the amino acid sequence set forth in SEQ ID NO:6, which shares 100% sequence identity to the fusion protein encoded by SEQ ID NO:1 of the instant application. While the '799 patent does not disclose the dosing schedules set forth in the instant claims, it is routine experimentation to optimize dosages and dosage schedules. The courts have determined that:

> "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller,* 220 F.2d 454, 454, 105 USPQ 223,235, (CCPA 1955).

Therefore, the claims are overlapping in scope.

17. Claims 1-20 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-15 of U.S. Patent No. 7,521,049. Although the conflicting claims are not identical, they are not patentably distinct from each other because claims 1-15 of the '049 patent are drawn to a method for treating an eye disorder, including age-related macular degeneration, diabetic retinopathy, choroidal neovascularization, vascular leak, and/or retinal edema, comprising administering a fusion polypeptide having the amino acid sequence set forth in SEQ ID NO:23, which shares 100% sequence identity to the fusion protein

encoded by SEQ ID NO:1 of the instant application. While the '049 patent does not disclose the dosing schedules set forth in the instant claims, it is routine experimentation to optimize dosages and dosage schedules. The courts have determined that:

> "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller,* 220 F.2d 454, 454, 105 USPQ 223,235, (CCPA 1955).

Therefore, the claims are overlapping in scope.

Summary

18. No claim is allowed.

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, **Joanne Hama**, can be reached on **(571) 272-2911**. 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.

/JON M LOCKARD/
Examiner, Art Unit 1647
June 15, 2015

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

=> DIS HIST

(FILE 'HOME' ENTERED AT 21:49:08 ON 13 JUN 2015)

FILE 'MEDLINE, SCISEARCH, EMBASE, BIOSIS' ENTERED AT 21:51:29 ON 13 JUN 2015

L1 3855 S (FLT1 OR VEGFR1 OR (VEGF (W) R1)) (P) ((FLK1 OR KDR OR VEGFR2
L2 67 S L1 AND ((CHIMER? OR FUSION) (P) VEGF)
L3 31 DUP REM L2 (36 DUPLICATES REMOVED)
L4 1488 S VEGF (3A) TRAP?
L5 1477 S VEGF (2A) TRAP?
L6 58 S L1 AND L5
L7 24 DUP REM L6 (34 DUPLICATES REMOVED)
L8 8 S (L3 OR L7) AND (EYE OR OCULAR OR MACULAR OR RETINA? OR CHO
E YANCOPOULOS G/AU
L9 1865 S E3-E9
L10 9 S L1 AND L9

Receipt date: 03/17/2015
Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	13940370
Filing Date	2013-07-12
First Named Inventor	George D. YANCOPOULOS
Art Unit	1647
Examiner Name	LOCKARD, Jon McClelland
Attorney Docket Number	REGN-008CIP

U.S.PATENTS Remove

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	7396664		2008-07-08	Daly et al.	

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Receipt date: 03/17/2015	Application Number	13940370
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2013-07-12
(Not for submission under 37 CFR 1.99)	First Named Inventor	George D. YANCOPOULOS
	Art Unit	1647
	Examiner Name	LOCKARD, Jon McClelland
	Attorney Docket Number	REGN-008CIP

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Receipt date: 03/17/2015	Application Number	13940370
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2013-07-12
	First Named Inventor	George D. YANCOPOULOS
	Art Unit	1647
	Examiner Name	LOCKARD, Jon McClelland
	Attorney Docket Number	REGN-008CIP

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☒ See attached certification statement.

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☐ A certification statement is not submitted herewith.

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	/Karl Bozicevic, Reg. No. 28,807/	Date (YYYY-MM-DD)	2015-03-17
Name/Print	Karl Bozicevic	Registration Number	28807

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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.

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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.

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Receipt date: 10/18/2013
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Doc description: Information Disclosure Statement (IDS) Filed

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	13940370
	Filing Date	2013-07-12
	First Named Inventor	YANCOPOULOS, GEORGE D.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-008CIP

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	2	20050260203		2005-11-24	Wiegand et al.	
	3	20060058234		2006-03-16	Daly et al.	
	4	20060172944		2006-08-03	Wiegand et al.	
/J.L./	5	20070190058		2007-08-16	Shams	

Receipt date: 10/18/2013	Application Number	13940370
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2013-07-12
(Not for submission under 37 CFR 1.99)	First Named Inventor	YANCOPOULOS, GEORGE D.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-008CIP

/J.L./	6	20030171320		2003-09-11	Guyer	

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/J.L./	1	00/75319	WO		2000-12-14	Regeneron Pharmaceuticals, Inc.		☐

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/J.L./	1	ANONYMOUS "Lucentis (rangibizymab injection) Intravitreal Injection" pp. 103 (June 2006)	☐
	2	DO et al., "An exploratory study of the safety, tolerability and bioactivity of a single intravitreal injection of vascular endothelial growth factor Trap-Eye in patients with diabetic macular oedema" Br J Opthamol. 93(2):144-1449 (February 2009)	☐
	3	DO et al., "The DA VINCI Study: phase 2 primary results of VEGF Trap-Eye in patients with diabetic macular edema" Opthamology 118(9):1819-1826 (September 2011)	☐
	4	THE EYETECH STUDY GROUP, "Anti-Vascular Endothelial Growth Factor Therapy for Subfoveal Choroidal Neovascularization Secondary to Age-related Macular Degeneration" American Academy of Ophthamology, 110 (5):979-986 (May 2003)	☐
/J.L./	5	HEIERet al., " rhuFab V2 (anti-VEGF Antibody) for Treatment of Exudative AMD" Symposium 8:Experimental and Emerging Treatments for Choroidal Neovascularization, 10 pp (2002)	☐

Receipt date: 10/18/2013	Application Number	13940370
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2013-07-12
(Not for submission under 37 CFR 1.99)	First Named Inventor	YANCOPOULOS, GEORGE D.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-008CIP

/J.L./	6	HEIER et al., "RhuFab V2 in Wet AMD - 6 Month Continued Improvement Following Multiple Intravitreal Injections" Invest Ophthalmol Vis Sci, 44:E-Abstract 972 (2003)	☐
	7	KRZYSTOLIK et al., "Prevention of Experimental Choroidal NEovascularization With Intravitreal Anti-Vascular Endothelial Growth Factor Antibody Fragment" Arch Ophthamol., 120:338-346 (Mar. 2002)	☐
	8	NGUYEN et al., "A Phase I Study of Intravitreal Vascular Endothelial Growth Factor Trap-Eye in Patients with Neovascular Age-Related Macular Degeneration" Opthamology, J.B. Lippincott Co., Philadelphia, PA, US, 116(11):2141-2148 (November 1, 2009)	☐
	9	NICHOLS, EARL R., "AAO: Ranibizumab (rhuRab) May Improve Vision in Age-Related Macular Degeneration" Doctor's Guide Global Edition, www.pslgroup.com/dg/23f2aa.htm, pp. 1-2 (November 24, 20013)	☐
	10	PAI et al., "Current concepts in intravitreal drug therapy for diabetic retinopathy" Saudi Journal of Opthamology 24(4):143-149 (June 30, 2010)	☐
	11	STEWART, "THe expanding role of vascular endothelial growth factor inhibitors in opthamology" Mayo Clin Proc. 87 (1):77-88 (January 2012)	☐
/J.L./	12	THOMAS REUTERS INTEGRITY "VEGF Trap-Eye final phase II results in age-related macular degeneration presented at 2008 Retina Society Meeting" (September 28, 2008)	☐

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EXAMINER SIGNATURE

Examiner Signature	/Jon Lockard/	Date Considered	05/30/2015

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Receipt date: 10/18/2013	Application Number	13940370
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2013-07-12
	First Named Inventor	YANCOPOULOS, GEORGE D.
	Art Unit	
	Examiner Name	
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☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

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	/Karl Bozicevic, Reg. No. 28,807/	Date (YYYY-MM-DD)	2013-10-18
Name/Print	Karl Bozicevic	Registration Number	28807

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.**

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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.

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13940370	YANCOPOULOS, GEORGE D.
	Examiner	Art Unit
	JON M LOCKARD	1647

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
NONE		6/13/2015	JML

SEARCH NOTES		
Search Notes	Date	Examiner
STIC Search of SEQ ID NOs:1-2. See sequence search results in SCORE.	6/13/2015	JML
EAST (USPAT, US-PGPUB, EPO, DERWENT): See attached search history.	6/13/2015	JML
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EAST Search History

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L1	4807	(flt1 or vegfr1 or (vegf adj r1)) same ((flk1 or kdr or vegfr2 or (vegf adj r2)) or (Flt4 vegfr3 or (vegf adj r3)))	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2015/06/13 21:59
L2	1152	l1 and ((chimer$ or fusion) same vegf)	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2015/06/13 21:59
L3	4709	(flt1 or vegfr1 or (vegf adj r1)) with ((flk1 or kdr or vegfr2 or (vegf adj r2)) or (Flt4 vegfr3 or (vegf adj r3)))	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2015/06/13 21:59
L4	1129	l3 and ((chimer$ or fusion) same vegf)	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2015/06/13 22:00
L5	416	l1 same ((chimer$ or fusion) same vegf)	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2015/06/13 22:00
L6	402	l3 same ((chimer$ or fusion) same vegf)	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2015/06/13 22:00
L7	211	l3 with ((chimer$ or fusion) with vegf)	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2015/06/13 22:00
L8	100	(l5 or l6) and ((eye or ocular or retina$ or macular) with disorder)	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2015/06/13 22:01
L9	6	(l5 or l6) same ((eye or ocular or retina$ or macular) with disorder)	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2015/06/13 22:01
L10	85	l7 and (((eye or ocular) with disorder) or (macular adj degeneration) or (diabetic adj retinopathy))	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2015/06/13 22:01
L11	363	yancopoulos-g$.in.	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2015/06/13 22:02
L12	10	l8 and l11	US-PGPUB; USPAT; EPO; DERWENT	OR	OFF	2015/06/13 22:02

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Appln Info | Contents | Petition Info | Atty/Agent Info | Continuity Data | Foreign Data | **Inventors** | Applicants | Address | Fees | Post Info | Pre Gra

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AMENDMENT UNDER 37 C.F.R. §1.111 Address to: Mail Stop: Amendment P.O. Box 1450 Alexandria, VA 22313-1450	Attorney Docket No.	REGN-008CIP
	Confirmation No.	1055
	First Named Inventor	YANCOPOULOS, GEORGE D.
	Application Number	13/940,370
	Filing Date	July 12, 2013
	Group Art Unit	1647
	Examiner Name	LOCKARD, JON MCCLELLAND
	Title: "Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders"	

Sir:

This amendment is responsive to the Office Action dated June 23, 2015, for which a three-month period for response was given, making this response due on or before September 23, 2015.

The claims begin on page 2 of this document.

The remarks begin on page 5 of this document.

In view of the amendments to the claims, and remarks put forth below, reconsideration and allowance are respectfully requested.

AMENDMENTS TO THE CLAIMS

1. (**Currently Amended**) A method for treating an angiogenic eye disorder in a patient, said method comprising sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist;

wherein each secondary dose is administered 2 to 4 weeks after the immediately preceding dose; and

wherein each tertiary dose is administered at least 8 weeks after the immediately preceding dose**;**

wherein the VEGF receptor-based chimeric molecule comprises (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130-231 of SEQ ID NO:2; and (3) a multimerization component comprising amino acids 232-457 of SEQ ID NO:2.

2. (Original) The method of claim 1, wherein only a single secondary dose is administered to the patient, and wherein the single secondary dose is administered 4 weeks after the initial dose of the VEGF antagonist.

3. (Original) The method of claim 1, wherein only two secondary doses are administered to the patient, and wherein each secondary dose is administered 4 weeks after the immediately preceding dose.

4. (Original) The method of claim 3, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

5. (Original) The method of claim 1, wherein at least 5 tertiary doses of the VEGF antagonist are administered to the patient, and wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered 8 or 12 weeks after the immediately preceding dose.

6. (Original) The method of claim 1, wherein the angiogenic eye disorder is selected from the group consisting of: age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization.

7. (Original) The method of claim 6, wherein the angiogenic eye disorder is age related

macular degeneration.

8.-11. (**Canceled**)

12. (Original) The method of claim 1, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

13. (Original) The method of claim 12, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

14. (Original) The method of claim 13, wherein the intraocular administration is intravitreal administration.

15. – 17. (**Canceled**)

18. (**Currently amended**) The method of claim ~~17~~ **14**, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

19. (Original) The method of claim 18, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

20. (Original) The method of claim 18, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

21. (**New**) A method for treating an angiogenic eye disorder in a patient, said method comprising sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist;

wherein each secondary dose is administered 2 to 4 weeks after the immediately preceding dose; and

wherein each tertiary dose is administered at least 8 weeks after the immediately preceding dose;

wherein the VEGF receptor-based chimeric molecule comprises VEGFR1R2-FcΔC1(a) encoded by the nucleic acid sequence of SEQ ID NO:1.

22. (**New**) The method of claim 21, wherein only a single secondary dose is administered to the patient, and wherein the single secondary dose is administered 4 weeks after the initial dose of the

VEGF antagonist.

23. (**New**) The method of claim 21, wherein only two secondary doses are administered to the patient, and wherein each secondary dose is administered 4 weeks after the immediately preceding dose.

24. (**New**) The method of claim 23, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

25. (**New**) The method of claim 21, wherein at least 5 tertiary doses of the VEGF antagonist are administered to the patient, and wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered 8 or 12 weeks after the immediately preceding dose.

26. (**New**) The method of claim 21, wherein the angiogenic eye disorder is selected from the group consisting of: age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization.

27. (**New**) The method of claim 24, wherein the angiogenic eye disorder is age related macular degeneration.

28. (**New**) The method of claim 21, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

29. (**New**) The method of claim 28, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

30. (**New**) The method of claim 29, wherein the intraocular administration is intravitreal administration.

31. (**New**) The method of claim 30, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

32. (**New**) The method of claim 31, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

33. (**New**) The method of claim 31, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

REMARKS

Formal Matters

Claims 1-7, and 12-14 and 18-33 are now pending in this application.

Claims 1 and 18 have been amended.

Claims 8-11 and 15-17 have been canceled without prejudice.

Claims 21-33 have been added.

The amendments to claim 1 are fully supported within originally pending now canceled claim 11.

The amendment to claim 18 is formal in nature and necessitated by the cancellation of claim 17 from the application.

New claim 21 is fully supported within originally pending claims 1 and 10.

Claims 22-27 are identical original claims 2-7 except that they are dependent on newly added claims.

New claims 28-30 are identical to original claims 12-14 except that they are dependent on newly added claims.

New claims 31-33 are identical to original claims 18-20 except that they are dependent on newly added claims.

No new matter has been added.

REJECTION UNDER 35 U.S.C. §112

Claims 1-9 and 12-20 were rejected under 35 U.S.C §112 first paragraph. The rejection was not applied against dependent claims 10 or 11.

Applicants do not acquiesce to the validity of the rejection. However, claim 1 has been amended to incorporate the limitations of previously pending now canceled dependent claim 11. In view of such, the rejection is believed to have been overcome or rendered moot in that the limitations of claim 11 are now included within independent claim 1. All the remaining claims 2-7, 12-14 and 18-20 are dependent directly or indirectly on claim 1. Accordingly, the rejection no longer applies to these claims.

New claim 21 is the independent form of original claims 1 and 10. In that claim 10 was not rejected under 35 U.S.C. §112 first paragraph, the rejection is not believed to be applicable to new claim 21.

All of claims 22-33 are dependent directly or indirectly on new claim 21. Accordingly, the rejection is not believed to be applicable to these claims.

In view of the above and the amended claims, the 35 U.S.C. §112 first paragraph rejection is believed to have been overcome or rendered moot.

DOUBLE PATENTING REJECTION

Claims 1-20 were rejected under the judicially created doctrine of obviousness-type double patenting over claims 1-5 of U.S. Patent No. 7,303,746; claims 1-6 of U.S. Patent No. 7,303,747; claims 1-11 of U.S. Patent No. 7,306,799; and claims 1-15 of U.S. Patent No. 7,521,049.

The rejections are respectfully traversed as applied and as they might be applied to the presently pending claims.

In support of the rejection, it is argued that the claims of the cited patents claim methods of treating eye disorders. Although the rejection points out that the patents do not disclose schedules set within the current claims, it is argued that where the general conditions of a claim are disclosed within the prior art, it is not inventive to discover optimum or workable ranges by routine experimentation.

Based on the working examples set forth in the present application, along with the endorsement of the present invention as set out in the attached peer reviewed publication, as well as the facts and reasoning provided below, the rejection should be reconsidered and withdrawn.

At the time of the invention the well accepted standard of care for the treatment of the neovascular (or wet) form of age-related macular degeneration (AMD) was to administer an antibody formulation (ranibizumab) by injection to the eye once per month (see the attached Heier et al. paper).

This treatment protocol is (1) expensive; (2) painful to the patient; (3) inconvenient for the patient as well as the patient's family; (4) psychologically and physically traumatic to the patient; and (5) subjects the patient to potential adverse effects such as infection with each treatment event.

Due to all the above factors (1-5) there was a need in the art for alternative treatment protocols whereby the treatment would be carried out with less inconvenience and reduced safety risks to the patient. However, until the present invention once a month treatment remained the standard of care.

There are virtually an infinite number of different treatment protocols that could be tested. A drug could be administered more frequently, or less frequently, relative to the accepted standard of care. Further, different variations in timing between dosing events are possible. Due to the virtually infinite number of combinations, applicants do not believe that the claimed treatment protocol is *prima facie* obvious in view of the prior art standard of care which is administration of the drug once per month. However, notwithstanding that position, any *prima facie* case of obviousness is overcome by the showing of improved unexpected results. Thus, while the rejection is citing case law which supports the

position that where the general conditions of a claim are disclosed within the prior art, it is not inventive to discover optimal ranges, the Examiner is aware that this case law is not applicable to situations where improved unexpected results are shown. Such results have been obtained and are described in the working examples of the present application and in the attached publication, portions of which are referred to below.

The attached Heier et al. paper published in December of 2012, and as such is not prior art with respect to the present application filed on January 11, 2012 and claiming priority to November 21, 2011.

The Heier et al. paper shows results of a treatment protocol of the type claimed on over 2,400 patients. The studies summarized in the Heier *et al.* paper correspond to the clinical trials disclosed in Example 4 of the present application which involve the use of the VEGF receptor-based chimeric molecule known as aflibercept or "VEGF Trap."[1] The results clearly show that by administering the VEGF antagonist in accordance with a dosage regimen as claimed in independent claims 1 and 21, it is possible to treat angiogenic eye disorders such as AMD while administering doses on a less frequent basis than previously thought possible. This provides enormous benefits to patients, reduces health care cost, reduces the pain and suffering of the patient, as well as the inconvenience to the patient and their family, and as such provides a major step forward in the treatment of patients suffering from angiogenic eye disorders, which is worthy of patent protection.

The attached Heier et al. article is a peer reviewed article published in "Ophthalmology" which describes the aforementioned clinical trial as follows:

> "Patients were randomized in a 1:1:1:1 ratio to the following regimens: 0.5 mg aflibercept every 4 weeks (0.5q4); 2 mg aflibercept every 4 weeks (2q4); 2mg aflibercept every 8 weeks (2q8) after 3 injections at week 0, 4 and 8 (to maintain masking, sham injections were given at the interim 4-week visits after week 8); or 0.5mg ranibizumab every 4 weeks (Rq4). Consecutively enrolled patients were assigned to treatment groups on the basis of predetermined central randomization scheme with balanced allocation, managed by an interactive voice response system. "

In the "primary end point analysis" section of the paper, it is indicated that the proportion of

[1] Aflibercept is a VEGF receptor-based chimeric molecule as defined in claims 1 and 21.

patients maintaining vision was similar among all treatment groups and this is dramatically shown within Figure 2. Thus, the results show that the treatment groups which were compared with the monthly treatment groups surprisingly did not obtain an inferior result. As such, the treatment protocol as encompassed by the presently pending independent claims 1 and 21 achieves results which are as good or better than the results obtained with monthly treatment.

Within the "Discussion" section of the Heier et al. paper, it is noted that the treatment group treated every two months achieved a visual acuity score within 0.3 letters of the group treated on a monthly basis. See also the results summarized in Table 1, page 15, of the present application. Thus, it is indicated that the treatment group which received the drug far less frequently than the monthly dosing arm achieved remarkably similar improvements without requiring the monthly monitoring and visits to the health care provider.

Similar remarkable results are shown in Example 5 of the present application, which illustrates an administration regimen encompassed by claims 1 and 21 (*i.e.*, 3 initial doses of VEGF Trap administered once every four weeks, followed by additional doses administered once every 8 weeks) for the effective treatment of diabetic macular edema (DME). As noted at paragraph [0065] of the present specification: "the administration of VEGFT to patients suffering from angiogenic eye disorders (*e.g.*, AMD and DME) at a frequency of once every 8 weeks, following a single initial dose and two secondary doses administered four weeks apart, resulted in significant prevention of moderate or severe vision loss or improvements in visual acuity."

An acknowledgement of the unexpected results of the administration regimen of the present invention is echoed in the Heier et al. paper, which points out that less frequent injections should also provide an ocular safety benefit, and that using fewer injections may substantially decrease the cumulative population risk of certain adverse events which can have a considerable impact considering the millions of injections given each year. For example, Heier et al. states on page 2546, middle left colum that:

> "The demonstration that monthly aflibercept provides similar efficacy and safety as the current approved standard of monthly ranibizumab is important, but the finding that remarkably similar improvement in vision and anatomic measures can be achieved with less than monthly intravitreal aflibercept injections and without requiring monthly monitoring visits provides an important advance for both patients and their treating physicians."

Moreover, the final paragraph of the Heier et al. paper reads as follows:

> "In conclusion, intravitreal aflibercept dosed monthly or every 2 months after 3 initial monthly doses resulted in similar visual and anatomic outcomes as ranibizumab dosed monthly, as well as similar safety and tolerability. Intravitreal aflibercept dosed every 2 months has the potential to provide patients, their families and clinicians the opportunity for the optimal vision gains and anatomic disease control they have come to expect from monthly ranibizumab, with a substantially decreased treatment and compliance burden, and a lower cumulative risk of injection-related adverse events."

Based on the above, it is clear that the claimed treatment protocol provides enormous advantages to patients. Further, in view of the disadvantages of carrying out the treatment on a once per month basis, there was a need in the art for alternative treatment protocols. However, this did not occur until the present invention and as such, the claimed treatment protocol is inventive above and beyond the inventions claimed within the patents cited in the obviousness type double patenting rejection. In view of such, those rejections should be reconsidered and withdrawn.

Applicants do not acquiesce to the *prima facie* obviousness of the claimed invention over the invention claimed within the cited patents. This is because there are virtually an infinite number of different possible treatment protocols. However, notwithstanding any *prima facie* case of obviousness, applicants have demonstrated improved and unexpected results, and based on such, the rejections should be reconsidered and withdrawn.

SUMMARY

All the claims but for claims 10 and 11, were rejected under 35 U.S.C. §112 first paragraph. Without acquiescing to the rejection, claim 1 was amended to incorporate the limitations of dependent claim 11 and new claim 21 incorporates the limitations of original claims 1 and 10. Thus, without acquiescing to the validity of the rejection, it has been rendered moot. The obviousness type double patenting rejection should be reconsidered in view of the enormous benefit provided by the treatment protocol claimed by applicants which treatment protocol clearly was not obvious to others skilled in the art.

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-008CIP.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 11 September 2015

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
Registration No. 28,807

Attachments: Heier et al. "RhuFab V2 in Wet AMD – 6 Month Continued Improvement Following Multiple Intravitreal Injections" Invest Opthalmol Vis Sci, 44:E-Abstract 972 (2003)

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Intravitreal Aflibercept (VEGF Trap-Eye) in Wet Age-related Macular Degeneration

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Objective: Two similarly designed, phase-3 studies (VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD [VIEW 1, VIEW 2]) of neovascular age-related macular degeneration (AMD) compared monthly and every-2-month dosing of intravitreal aflibercept injection (VEGF Trap-Eye; Regeneron, Tarrytown, NY, and Bayer HealthCare, Berlin, Germany) with monthly ranibizumab.

Design: Double-masked, multicenter, parallel-group, active-controlled, randomized trials.

Participants: Patients (n = 2419) with active, subfoveal, choroidal neovascularization (CNV) lesions (or juxtafoveal lesions with leakage affecting the fovea) secondary to AMD.

Intervention: Patients were randomized to intravitreal aflibercept 0.5 mg monthly (0.5q4), 2 mg monthly (2q4), 2 mg every 2 months after 3 initial monthly doses (2q8), or ranibizumab 0.5 mg monthly (Rq4).

Main Outcome Measures: The primary end point was noninferiority (margin of 10%) of the aflibercept regimens to ranibizumab in the proportion of patients maintaining vision at week 52 (losing <15 letters on Early Treatment Diabetic Retinopathy Study [ETDRS] chart). Other key end points included change in best-corrected visual acuity (BCVA) and anatomic measures.

Results: All aflibercept groups were noninferior and clinically equivalent to monthly ranibizumab for the primary end point (the 2q4, 0.5q4, and 2q8 regimens were 95.1%, 95.9%, and 95.1%, respectively, for VIEW 1, and 95.6%, 96.3%, and 95.6%, respectively, for VIEW 2, whereas monthly ranibizumab was 94.4% in both studies). In a prespecified integrated analysis of the 2 studies, all aflibercept regimens were within 0.5 letters of the reference ranibizumab for mean change in BCVA; all aflibercept regimens also produced similar improvements in anatomic measures. Ocular and systemic adverse events were similar across treatment groups.

Conclusions: Intravitreal aflibercept dosed monthly or every 2 months after 3 initial monthly doses produced similar efficacy and safety outcomes as monthly ranibizumab. These studies demonstrate that aflibercept is an effective treatment for AMD, with the every-2-month regimen offering the potential to reduce the risk from monthly intravitreal injections and the burden of monthly monitoring.

Financial Disclosure(s): Proprietary or commercial disclosure may be found after the references.
Ophthalmology 2012;119:2537–2548 © 2012 by the American Academy of Ophthalmology.



**Group members listed online in Appendix 1 (http://aaojournal.org).*

Age-related macular degeneration (AMD) is a leading cause of vision loss and blindness in industrialized countries.[1] The most severe vision loss occurs in the neovascular (or wet) form of AMD, involving choroidal neovascularization (CNV) and associated retinal edema. Early treatments for CNV (laser ablation, photodynamic therapy with verteporfin), although clearly better than no treatment at all, decreased severe vision loss rather than truly stabilizing vision or resulting in clinically significant improvements in visual acuity.[2–4] The suggestion that vascular endothelial growth factor (VEGF) might be driving the CNV and associated edema seen in AMD led to a paradigm shift with the success of the first anti-VEGF therapy, pegaptanib sodium.[5,6] Monthly intravitreal injections of 0.5 mg ranibizumab, a humanized monoclonal antibody fragment that blocks VEGF, not only prevent vision loss in most patients but also lead to significant visual gain in approximately one-third.[7,8] The risk of rare but serious adverse events resulting from the intravitreal procedure, together with the significant burden of making monthly visits to their retinal specialist, have led to extensive efforts to decrease injection and monitoring frequency. However, fixed quarterly[9,10] or "as needed" (pro re nata [PRN]) dosing regimens,[11,12] without requiring monthly monitoring visits, were not effective at maintaining vision.

The Comparison of AMD Treatments Trials (CATT)[13] recently compared monthly ranibizumab with monthly

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Published by Elsevier Inc.

ISSN 0161-6420/12/$–see front matter
http://dx.doi.org/10.1016/j.ophtha.2012.09.006

bevacizumab, as well as with PRN regimens that required monthly monitoring visits during which treatment decisions primarily were made on the basis of anatomic criteria. Monthly bevacizumab resulted in mean best-corrected visual acuity (BCVA) gains (8.0 letters) similar to those for monthly ranibizumab (8.5 letters), whereas PRN ranibizumab yielded a mean BCVA gain of 1.7 letters less than that of the monthly standard (with a confidence interval [CI] extending to 4.7 letters below) that achieved noninferiority, and PRN bevacizumab yielded a mean BCVA gain 2.6 letters below the monthly standard (with a CI extending to 5.9 letters below) that did not achieve noninferiority. In the CATT, monthly bevacizumab and both PRN regimens were significantly worse than monthly ranibizumab in terms of the proportion of patients who had fluid-free retinas on optical coherence tomography (OCT). Although CIs were not provided for monthly and PRN regimens, switching from monthly to PRN regimens in the second year of the CATT resulted in a significant worsening of BCVA and retinal thickness, as well as a significant decrease in the proportion of patients without retinal fluid.[14] The "alternative treatments to Inhibit VEGF in Age-related choroidal Neovascularization" (IVAN) study also found that the mean foveal retinal thickness and the percentage of patients with fluorescein leakage were significantly higher with the PRN regimen compared with the monthly regimen.[15] In the HARBOR study (Invest Ophthalmol Vis Sci 2012;53:E-Abstract 3677), PRN regimens of both the approved 0.5 mg dose and the higher 2 mg dose of

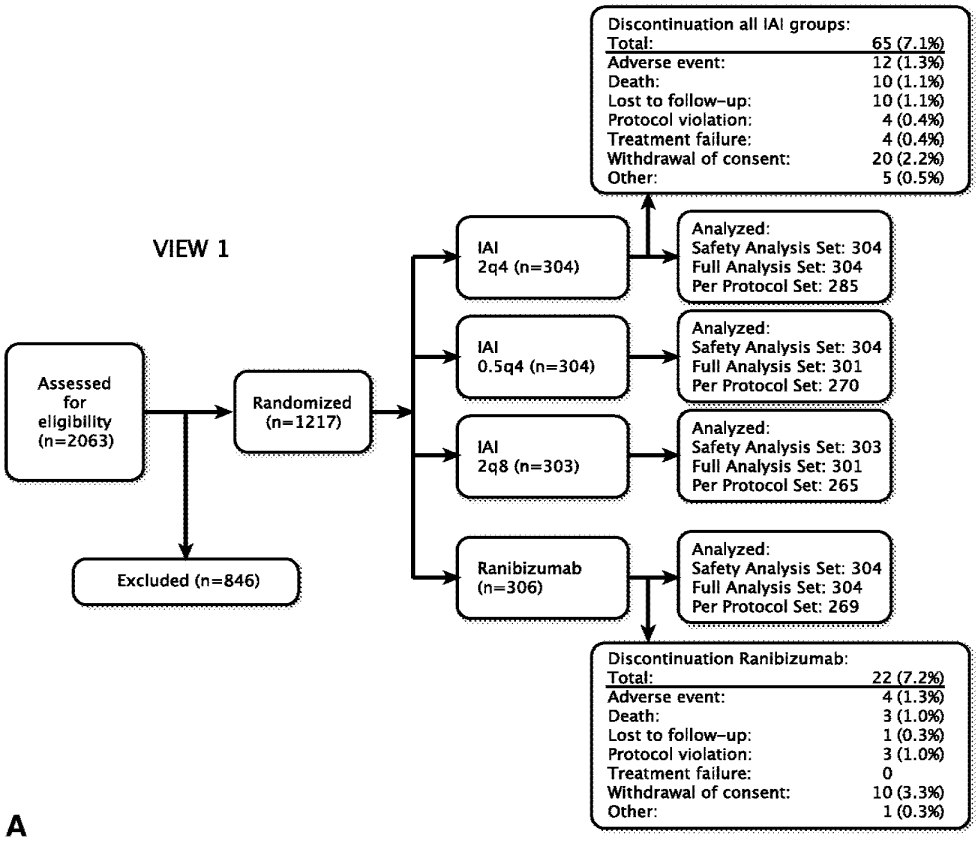


Figure 1. Flowcharts describing treatment allocation and patient disposition in VIEW 1 **(A)** and VIEW 2 **(B)**. In both VIEW 1 and VIEW 2 studies, the most common reason for patients to be screened but not randomized was ineligibility based on angiographic characteristics as identified by the reading center. The second most common reason was visual acuity out of range. Discontinuations are those that occurred from the study. Two milligrams intravitreal aflibercept every 2 months (2q8) dosing was performed after 3 initial monthly doses. The numbers of patients who prematurely discontinued study medication in the 2q4, 0.5q4, 2q8, and Rq4 groups were 16 (5.3%), 30 (9.9%), 30 (9.9%), and 27 (8.8%), respectively, in VIEW 1; and 37 (11.8%), 45 (14.5%), 33 (10.5%), and 33 (10.9%), respectively, in VIEW 2. In VIEW 1, 1089 patients were included in the per protocol set (PPS), with 92.6% to 96.1% completing week-52 visual acuity assessment. A total of 128 patients were not included in the PPS for the following reasons (in order of occurrence): missed 2 consecutive injections before ninth injection, major protocol deviation, received <9 injections, had <9 assessments, no baseline assessments, no post-baseline assessments. In VIEW 2, 1081 patients were included in the PPS with 95.9% to 97.8% completing week-52 visual acuity assessment. A total of 159 patients were not included in the PPS for the following main reasons: missed 2 consecutive injections before ninth injection, major protocol deviation, received <9 injections, had <9 assessments, no baseline assessments, no post-baseline assessments, unmasking by investigator or Global Pharmacovigilance. 0.5q4 = 0.5 mg IAI monthly; 2q4 = 2 mg IAI monthly; 2q8 = 2 mg IAI every 2 months after 3 initial monthly doses; IAI = intravitreal aflibercept injection.

ranibizumab did not achieve noninferiority compared with monthly ranibizumab, with the 0.5 mg PRN regimen yielding a mean BCVA gain 2.0 letters below the monthly standard (with a CI extending to 4.5 letters below). Of note, just like the CATT PRN regimens, the HARBOR PRN regimens still depended on monthly monitoring visits. Thus, there remains a need for new therapies that will provide equivalent efficacy and anatomic disease control to monthly ranibizumab, while reducing the risk of monthly injections and the burden of mandatory monthly monitoring visits.

Intravitreal aflibercept injection (IAI) (previously known in the scientific literature as VEGF Trap-Eye, Regeneron, Tarrytown, NY, and Bayer HealthCare, Berlin, Germany) is a soluble decoy receptor fusion protein[16,17] that is specifically purified and formulated for intraocular injection. Intravitreal aflibercept at doses of 0.5 mg and 2 mg provided the most robust outcomes in the Clinical Evaluation of Antiangiogenesis in the Retina Intravitreal Trial Phase 2 (CLEAR-IT 2) study after 4 monthly administrations followed by PRN dosing to week 52.[18] The binding affinity of intravitreal aflibercept to VEGF is substantially greater than that of bevacizumab or ranibizumab.[17] The greater affinity could translate into a higher efficacy or, as predicted by a mathematic model, into a substantially longer duration of action in the eye,[19] allowing for less frequent dosing, as supported by early clinical trials.[18,20] In this article, we report the first-year results of 2 phase 3 studies comparing intravitreal aflibercept, monthly or every 2 months, with monthly ranibizumab.

Materials and Methods

Study Design

The "VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD" studies (VIEW 1 and VIEW 2) were similarly designed, prospective, double-masked, multinational, parallel-group, active-controlled, randomized clinical trials. The investigators from the VIEW 1 and VIEW 2 studies are listed in Appendix 1, available at http://aaojournal.org. Patients in VIEW 1 (registered at www.clinicaltrials.gov on July 31, 2007; NCT00509795. Accessed August 8, 2012) were randomized at 154 sites in the United States and Canada. Patients in VIEW 2 (registered at www.clinicaltrials.gov on March 12, 2008; NCT00637377. Accessed August 8, 2012) were randomized at 172 sites in Europe, the Middle East, Asia-Pacific, and Latin America; the last patient in both studies completed 52 weeks in September 2010. The study protocols were approved by institutional review boards or ethics committees for each clinical site; all participants provided written informed consent. All the US study sites complied with the Health Insurance

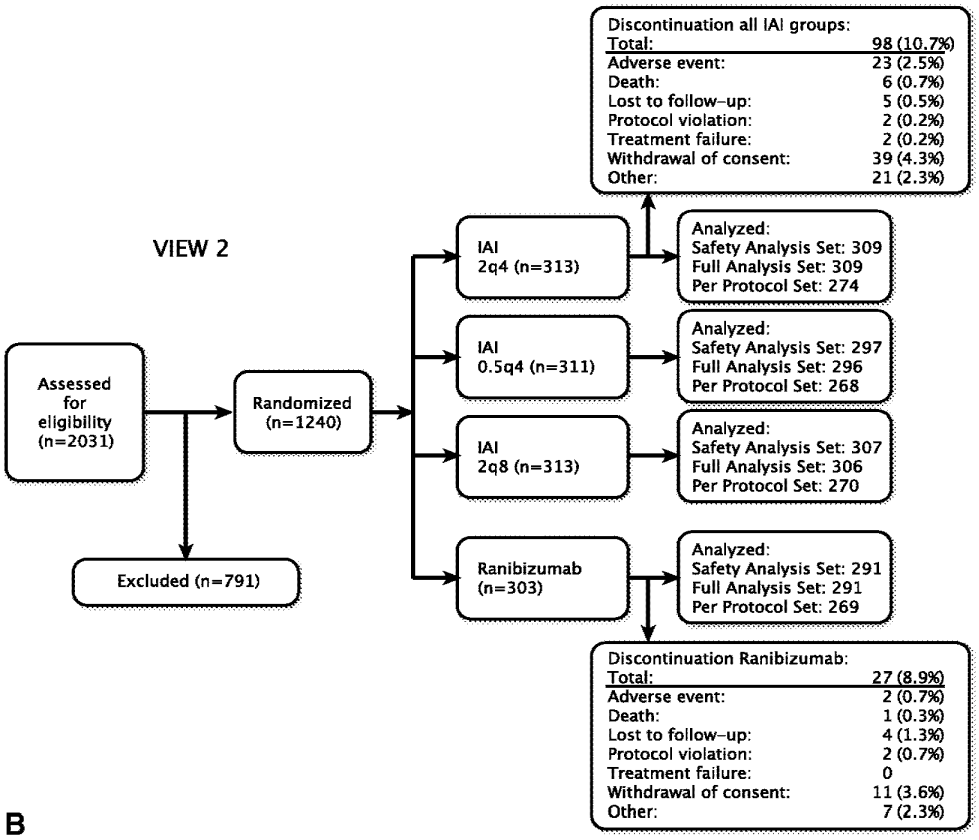


Figure 1. (Continued.)

Portability and Accountability Act. The 52-week outcomes are reported.

Participants

Inclusion and exclusion criteria were designed to maintain constancy with the pivotal trials for the reference drug ranibizumab, consistent with regulatory guidelines for noninferiority studies, and included (1) age ≥50 years with active subfoveal CNV lesions (any subtype) secondary to AMD; juxtafoveal lesions with leakage affecting the fovea also were allowed; (2) CNV comprising at least 50% of total lesion size; and (3) BCVA between 73 and 25 Early Treatment Diabetic Retinopathy Study chart (ETDRS) letters (20/40–20/320 Snellen equivalent). Patients with prior treatment for AMD (including an investigational agent or anti-VEGF therapy) in the study eye were excluded. Eligibility was determined using fluorescein angiography at the reading center. Complete eligibility criteria are shown in Appendix 2 (available at http://aaojournal.org).

Treatment Groups and Randomization

Patients were randomized in a 1:1:1:1 ratio to the following regimens: 0.5 mg aflibercept every 4 weeks (0.5q4); 2 mg aflibercept every 4 weeks (2q4); 2 mg aflibercept every 8 weeks (2q8) after 3 injections at week 0, 4, and 8 (to maintain masking, sham injections were given at the interim 4-week visits after week 8); or 0.5 mg ranibizumab every 4 weeks (Rq4). Consecutively enrolled patients were assigned to treatment groups on the basis of a predetermined central randomization scheme with balanced allocation, managed by an interactive voice response system.

End Points and Statistical Analyses

The primary end point analysis, noninferiority margins, and definition of "clinical equivalence" were established in discussion with the Food and Drug Administration (FDA) (as part of a Special Protocol Assessment), European Medicines Agency, Pharmaceutical and Medical Device Agency and other regulatory authorities, with the intent of maintaining constancy with the previous ranibizumab pivotal trials[7,8] and preserving the majority of the treatment effect demonstrated in these trials. The primary end point analysis was noninferiority of the intravitreal aflibercept regimens to ranibizumab in the proportion of patients maintaining vision at week 52 (losing <15 ETDRS letters; per protocol data set) in each study. A noninferiority margin of 10% in the individual studies was chosen to preserve approximately two-thirds of the ranibizumab effect for prevention of moderate vision loss (loss of <15 letters) demonstrated in pivotal ranibizumab studies,[7,8] using the 2 CI approach. The FDA suggested that a margin of 5% could determine clinical equivalence. Thus, the margin of 10% was used for assessing noninferiority, and the margin of 5% was used for assessing clinical equivalence. The prespecified analysis plan also included a prospectively planned integrated analysis combining the 2 VIEW studies; in this integrated analysis, the European Medicines Agency/Committee for Medicinal Products for Human Use requested a noninferiority margin of 7%. In the individual studies, the primary end point was assessed by a prespecified hierarchical testing sequence of noninferiority to ranibizumab with the sequence of aflibercept 2q4, 0.5q4, and then 2q8 to control the 5% (4.9% for VIEW 1) overall type I error while maintaining a 5% significance level (4.9% for VIEW 1) for each individual comparison (see Appendices 3 and 4 for details of the statistical analysis, available at http://aaojournal.org). If all aflibercept groups demonstrated noninferiority to ranibizumab for the primary end point, additional comparisons with ranibizumab were prespecified regarding the secondary end points, also using a hierarchical testing sequence in which each secondary end point was tested for superiority of aflibercept over ranibizumab. Prespecified secondary efficacy variables compared baseline and 52-week data regarding mean change in BCVA; gaining ≥15 letters; change in total National Eye Institute 25-Item Visual Function Questionnaire (NEI VFQ-25) score; and change in CNV area on fluorescein angiography. Anatomic measures included retinal thickness and persistent fluid as assessed by OCT. Change in BCVA also was assessed as part of the prospectively planned prespecified integrated analysis combining the 2 studies.

The full analysis set included all randomized patients who received any study medication and had a baseline and at least 1 post-baseline BCVA assessment. The per protocol set (PPS) included all patients in the full analysis set who (1) received at least 9 doses of study drug and attended at least 9 scheduled visits during the first year, (2) had not missed 2 consecutive injections before administration of the ninth injection (per patient), and (3) did not have major protocol violations. Sham injections were counted as doses administered for the purpose of defining the PPS. The PPS included patients who discontinued the study because of treatment failure, without a major protocol deviation, at any time during the first 52 weeks (even if they met points 1 and 2 above). These patients were considered nonresponders for the primary end-point analysis. The last observation carried forward (LOCF) approach was used to impute missing values. When indicated, the robustness of analysis results was assessed by using the observed case or completers' data. A completer was defined as a patient who received treatment for at least 9 months and had efficacy data for at least 9 months during the 52 weeks of study. The missing values for completers also were imputed using the LOCF approach.

Schedule of Visits and Assessments

Patients were examined on the day of treatment initiation and every 4 weeks thereafter through 52 weeks, as well as 1 week after first treatment for safety assessment (subsequent safety assessments occurred by telephone). Each 4-week visit included BCVA assessment and anterior/posterior segment examination (with intraocular pressure determination) before injection (active or sham) and posterior segment examination with intraocular pressure determination 30 to 60 minutes after injection. For the 2q8 treatment group, no treatment decisions were made at the interim monthly visits. The NEI VFQ-25 assessment occurred at screening and weeks 12, 24, 36, and 52. Adverse events were recorded at every visit.

Imaging Assessments

Fundus photography and fluorescein angiography were performed at screening and weeks 24 and 52, and evaluated by an independent center (Digital Angiography Reading Center, New York). Optical coherence tomography was performed using time domain Stratus machines (Carl Zeiss Meditec, Jena, Germany) and evaluated by an independent center (VIEW 1: OCT Reading Center at Duke, Durham, NC; VIEW 2: Vienna Reading Center, Austria). Visual acuity examiners were certified to ensure consistent measurement of BCVA. In VIEW 1, OCT was performed at screening, at the treatment initiation visit, and at weeks 4, 12, 24, 36, and 52

(and was optional at the investigators' discretion at other study visits). In VIEW 2, OCT was performed at every study visit. Areas of visible CNV (classic or occult) were identified when angiographic analyses showed evidence of late leakage or pooling of dye.

Masking

Patients were masked as to treatments. An unmasked investigator performed the study drug or sham injection. An unmasked investigator also was responsible for the receipt, tracking, preparation, destruction, and administration of study drug, as well as safety assessments both pre- and post-dose. A separate masked physician assessed adverse events and supervised the masked assessment of efficacy. All other study site personnel were masked to treatment assignment by separating study records or masked packaging. Optical coherence tomography technicians and visual acuity examiners remained masked relative to treatment assignment. Intravitreal aflibercept and sham kits were packaged identically. Lucentis (Genentech Inc, South San Francisco, CA) was obtained commercially but only prepared and delivered by unmasked personnel at the sites.

Results

Patient Disposition, Baseline Characteristics, and Exposure

The disposition of patients is shown in Figure 1A-B. In VIEW 1, 1217 patients were randomized, with 91.1% to 96.4% of patients completing 52 weeks. In VIEW 2, 1240 patients were randomized, with 88.1% to 91.1% completing 52 weeks. Baseline demographics and disease characteristics were evenly balanced among all treatment groups (Table 1). The mean number of active injections received by patients in all monthly treatment arms, which were scheduled to receive 13 monthly injections, was 12.1 to 12.5 in VIEW 1 and 12.2 to 12.4 in VIEW 2. The aflibercept every-2-month groups, scheduled to receive 3 initial monthly injections followed by 5 active injections over the next 10 months, received an average of 7.5 active injections in VIEW 1 and in VIEW 2.

Primary End Point Analysis

In both studies, the proportion of patients maintaining vision was similar among all treatment groups in the prespecified per-protocol analysis and the full analysis set (Table 2). All aflibercept groups achieved statistical noninferiority compared with monthly ranibizumab, with the CIs of the difference between ranibizumab and

Table 1. Patient Demographics and Baseline Characteristics

	VIEW 1				VIEW 2			
	Ranibizumab	*Intravitreal Aflibercept*			*Ranibizumab*	*Intravitreal Aflibercept*		
	0.5q4	*2q4*	*0.5q4*	*2q8*	*0.5q4*	*2q4*	*0.5q4*	*2q8*
N (full analysis set)	304	304	301	301	291	309	296	306
Age, yrs (mean ± SD)	78.2±7.6	77.7±7.9	78.4±8.1	77.9±8.4	73.0±9.0	74.1±8.5	74.7±8.6	73.8±8.6
Race								
White	296 (97.4)	295 (97.0)	291 (96.7)	287 (95.3)	213 (73.2)	226 (73.1)	219 (74.0)	217 (70.9)
Black	1 (0.3)	1 (0.3)	0	1 (0.3)	1 (0.3)	0	1 (0.3)	2 (0.7)
Asian	0	3 (1.0)	5 (1.7)	4 (1.3)	60 (20.6)	67 (21.7)	61 (20.6)	69 (22.5)
Other	7 (2.3)	5 (1.6)	5 (1.7)	9 (3.0)	17 (5.8)	16 (5.2)	15 (5.1)	18 (5.9)
Sex								
Men, n (%)	132 (43.4)	110 (36.2)	134 (44.5)	123 (40.9)	122 (41.9)	133 (43.0)	149 (50.3)	131 (42.8)
Women, n (%)	172 (56.6)	194 (63.8)	167 (55.5)	178 (59.1)	169 (58.1)	176 (57.0)	147 (49.7)	175 (57.2)
Baseline ETDRS BCVA (mean ± SD)	54.0±13.4	55.2±13.2	55.6±13.1	55.7±12.8	53.8±13.5	52.8±13.9	51.6±14.2	51.6±13.9
Proportion of patients with ≥20/40 BCVA, % (n)	4.3% (13)	4.9% (15)	6.3% (19)	6.6% (20)	2.7% (8)	2.6% (8)	5.4% (16)	3.3% (10)
CNV area, mm^2 (mean ± SD)	6.53±5.2	6.59±5.1	6.49±4.5	6.57±5.1	7.59±5.3	8.25±5.8	7.70±5.3	7.75±5.5
Lesion type								
Predominantly classic, n (%)	82 (27.0)	87 (28.6)	81 (26.9)	71 (23.6)	70 (24.1)	72 (23.3)	80 (27.0)	88 (28.8)
Minimally classic, n (%)	101 (33.2)	105 (34.5)	97 (32.2)	110 (36.5)	104 (35.7)	112 (36.2)	103 (34.8)	106 (34.6)
Occult, n (%)	115 (37.8)	110 (36.2)	121 (40.2)	118 (39.2)	116 (39.9)	123 (39.8)	113 (38.2)	110 (35.9)
Patients with juxtafoveal lesions, n (%)	15 (4.9)	13 (4.3)	17 (5.6)	17 (5.6)	20 (6.9)	15 (4.9)	11 (3.7)	14 (4.6)
Lesion size, mm^2 (mean ± SD)	6.99±5.5	6.98±5.4	6.95±4.7	6.89±5.2	8.01±5.7	8.72±6.1	8.17±5.5	8.22±5.9
Central retinal thickness, μm (mean ± SD)	315.3±108.3	313.6±103.4	313.2±106.0	324.4±111.2	325.9±110.9	334.6±119.8	326.5±116.5	342.6±124.0
Baseline NEI VFQ-25 scores (mean ± SD)	71.8±17.2	70.4±16.6	71.1±17.8	69.6±16.8	72.9±19.1	70.3±19.4	74.0±18.2	71.3±19.1

0.5q4 = 0.5 mg monthly; 2q4 = 2 mg monthly; 2q8 = 2 mg every 2 months after 3 initial monthly doses; BCVA = best-corrected visual acuity; CNV = choroidal neovascularization; ETDRS = Early Treatment Diabetic Retinopathy Study; NEI VFQ-25 = National Eye Institute 25-Item Visual Functioning Questionnaire; SD = standard deviation.

Table 2. Prespecified Efficacy

	VIEW 1			
	Ranibizumab	*Intravitreal Aflibercept*		
	0.5q4	*2q4*	*0.5q4*	*2q8*
Primary end point				
N (PPS)	269	285	270	265
Proportion maintaining vision (losing <15 ETDRS letters), % (n)	94.4% (254)	95.1% (271)	95.9% (259)	95.1% (252)
N (full analysis set)	304	304	301	301
Proportion maintaining vision (losing <15 ETDRS letters, LOCF), % (n)	93.8% (285)	95.1% (289)	95.0% (286)	94.4% (284)
Secondary end points				
N (full analysis set)	304	304	301	301
Change in ETDRS BCVA (mean ± SD)	8.1±15.3	10.9±13.8	6.9±13.4	7.9±15.0
LS mean difference between IAI and ranibizumab (95% CI)*		3.15 (0.92 to 5.37)	−0.80 (−3.03 to 1.43)	0.26 (−1.97 to 2.49)
Proportion gaining ≥15 ETDRS letters, % (n)	30.9% (94)	37.5% (114)	24.9% (75)	30.6% (92)
LS mean difference between IAI and ranibizumab (95% CI)*		6.58 (−0.98 to 14.14)	−6.00 (−13.17 to 1.16)	−0.36 (−7.74 to 7.03)
Change in CNV area, mm^2 (mean ± SD)	−4.2±5.6	−4.6±5.5	−3.5±5.3	−3.4±6.0
LS mean difference between IAI and ranibizumab (95% CI)*		−0.33 (−1.04 to 0.38)	0.71 (−0.01 to 1.42)	0.86 (0.15–1.58)
Change in total NEI VFQ-25 score (mean ± SD)	4.9±14.0	6.7±13.5	4.5±11.9	5.1±14.7
LS mean difference between IAI and ranibizumab (95% CI)*		1.28 (−0.73 to 3.28)	−0.67 (−2.69 to 1.35)	−0.60 (−2.61 to 1.42)
Exploratory end point				
Change in central retinal thickness, μm (mean ± SD)	−116.8±109.0	−116.5±98.4	−115.6±104.1	−128.5±108.5
Post hoc end point†				
Proportion with dry retina (absence of cystic intraretinal edema and subretinal fluid on OCT), % (n)	63.6% (171)	64.8% (184)	56.7% (148)	63.4% (168)

0.5q4 = 0.5 mg monthly; 2q4 = 2 mg monthly; 2q8 = 2 mg every 2 months after 3 initial monthly doses; BCVA = best-corrected visual acuity; aflibercept injection; LOCF = last observation carried forward; LS = least-squares; NEI VFQ-25 = National Eye Institute 25-Item Visual
*95.1% CI for VIEW 1.
†Observed case.

each aflibercept group within the prespecified 10% margin (Fig 2), and the point estimates of the differences in means favoring the aflibercept groups in all cases. All the aflibercept regimens also met the prespecified 7% noninferiority margin in the prespecified integrated analysis combining the 2 VIEW studies, as well as the prespecified 5% margin for clinical equivalence compared with ranibizumab in the individual VIEW studies. Moreover, the results of multiple imputation analyses were consistent with those using the LOCF.

Mean Changes in Best-Corrected Visual Acuity and Other Visual Acuity End Points

The mean change in BCVA was a clinically important secondary end point in both studies. On the basis of the hierarchical testing sequence, only the aflibercept 2q4 group was statistically superior to ranibizumab, and only in VIEW 1, with a gain of +10.9 versus +8.1 letters (Table 2). Small numeric differences between treatment groups in one study at any given timepoint were not reproduced in the other study, suggesting that they reflected random variability even in groups of this size (Fig 3A, B); this interpretation was supported by a prespecified integrated analysis that combined the 2 studies (Fig 3C), showing similar visual acuity scores across the entire 52-week study for all treatment groups. All groups behaved similarly in this integrated analysis (Fig 3C), with rapid increases in mean visual acuity after the first injection followed by incremental gains that were durable and maintained through week 52. Regardless of whether the analysis was by LOCF, by multiple imputations, by assessing completers, or by using actual observed data, intravitreal aflibercept dosed every 2 months achieved a mean visual acuity score within 0.3 letters of monthly ranibizumab in the integrated analysis, with a CI of less than 2 letters (Fig 3C, inset).

In both studies, the secondary end point of proportions of patients gaining ≥15 ETDRS letters from baseline to week 52 was similar in all treatment groups (Table 2), as were other exploratory categoric measures of visual outcome (Appendix 5, available at http://aaojournal.org). Likewise, vision-related quality of life, assessed by the change of total score of the NEI VFQ-25, improved in all groups in both studies (Table 2).

Key Anatomic Measures

In both studies, all groups demonstrated a comparable decrease in the secondary end point of change in area of active CNV

Outcomes at Week 52

	VIEW 2			
	Ranibizumab	*Intravitreal Aflibercept*		
	0.5q4	*2q4*	*0.5q4*	*2q8*
Primary end point				
N (PPS)	269	274	268	270
Proportion maintaining vision (losing <15 ETDRS letters), % (n)	94.4% (254)	95.6% (262)	96.3% (258)	95.6% (258)
N (full analysis set)	291	309	296	306
Proportion maintaining vision (losing <15 ETDRS letters, LOCF), % (n)	94.8% (276)	94.5% (292)	95.3% (282)	95.4% (292)
Secondary end points				
N (full analysis set)	291	309	296	306
Change in ETDRS BCVA (mean ± SD)	9.4±13.5	7.6±12.6	9.7±14.1	8.9±14.4
LS mean difference between IAI and ranibizumab (95% CI)*		−1.95 (−4.10 to 0.20)	−0.06 (−2.24 to 2.12)	−0.90 (−3.06 to 1.26)
Proportion gaining ≥15 ETDRS letters, % (n)	34.0% (99)	29.4% (91)	34.8% (103)	31.4% (96)
LS mean difference between IAI and ranibizumab (95% CI)*		−4.57 (−12.02 to 2.88)	0.78 (−6.91 to 8.46)	−2.65 (−10.18 to 4.88)
Change in CNV area, mm^2 (mean ± SD)	−4.2±5.9	−6.0±6.1	−4.2±6.1	−5.2±5.9
LS mean difference between IAI and ranibizumab (95% CI)*		−1.18 (−1.98 to −0.38)	0.17 (−0.63 to 0.97)	−0.73 (−1.53 to 0.07)
Change in total NEI VFQ-25 score (mean ± SD)	6.3±14.8	4.5±15.0	5.1±13.7	4.9±14.7
LS mean difference between IAI and ranibizumab (95% CI)*		−2.79 (−4.90 to −0.68)	−0.93 (−3.07 to 1.20)	−1.95 (−4.07 to 0.17)
Exploratory end point				
Change in central retinal thickness, μm (mean ± SD)	−138.5±122.2	−156.8±122.8	−129.8±114.8	−149.2±119.7
Post hoc end point†				
Proportion with dry retina (absence of cystic intraretinal edema and subretinal fluid on OCT), % (n)	60.4% (162)	80.3% (220)	63.9% (170)	71.9% (197)

CNV = choroidal neovascularization; CI = confidence interval; ETDRS = Early Treatment Diabetic Retinopathy Study; IAI = intravitreal Functioning Questionnaire; OCT = optical coherence tomography; PPS = per protocol set; SD = standard deviation.

(Table 2). Likewise, all aflibercept groups in both studies had reductions in central retinal thickness similar to those for monthly ranibizumab as assessed by OCT, with a large and rapid reduction evident by week 4 (with retinal thickness approaching normal levels) that was maintained to week 52 (Table 2, Fig 4). Minor fluctuations in central retinal thickness were seen in the 2q8 group after sham injections in the VIEW 2 study; these fluctuations attenuated over time, starting at 17 μm and decreasing to 8 μm over the year, with no apparent negative impact on visual acuity outcomes.

Because of the inability of other regimens in the CATT[13] to match the retinal thickness and retinal fluid improvements seen with monthly ranibizumab, a post hoc analysis was performed to determine the percentage of patients who had fluid-free retinas, which were defined, on OCT, by the absence of both cystic intraretinal edema and subretinal fluid. All intravitreal aflibercept groups were similar to the monthly ranibizumab group in terms of this end point, with numerically higher percentages of dry retinas seen in the 2q4 and 2q8 regimens largely driven by VIEW 2 (Table 2; Appendix 6, available at http://aaojournal.org). Integrated analysis combining both studies for proportions of patients with dry retinas for ranibizumab and the aflibercept regimens of 2q4, 0.5q4, and 2q8 showed percentages of 62.0%, 72.4%, 60.3%, and 67.7%, respectively.

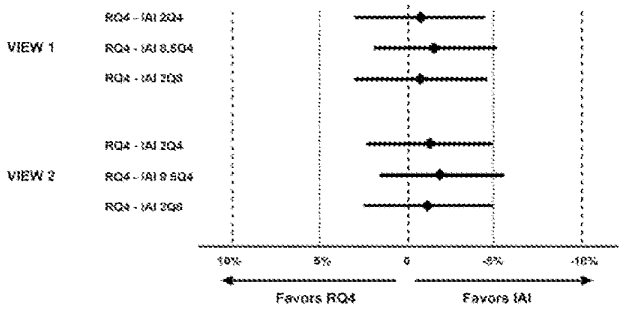


Figure 2. Difference in proportions of patients who maintained vision (losing <15 Early Treatment Diabetic Retinopathy Study [ETDRS] letters) at week 52 in the VIEW studies (per protocol set [PPS]). The *diamond symbol* denotes the difference between the treatment arms, and the *horizontal bars* indicate 95% confidence interval (CI) range. The CI within the left 10% (*dashed vertical lines*) indicates that all intravitreal aflibercept arms were noninferior to ranibizumab. The CI within the left 5% (*dotted vertical line*) indicates clinical equivalence to ranibizumab. The last observation carried forward (LOCF) was used for imputing the missing values. RQ4 = 0.5 mg ranibizumab monthly; 0.5Q4 = 0.5 mg IAI monthly; 2Q4 = 2 mg IAI monthly; 2Q8 = 2 mg IAI every 2 months after 3 initial monthly doses; IAI = intravitreal aflibercept injection.

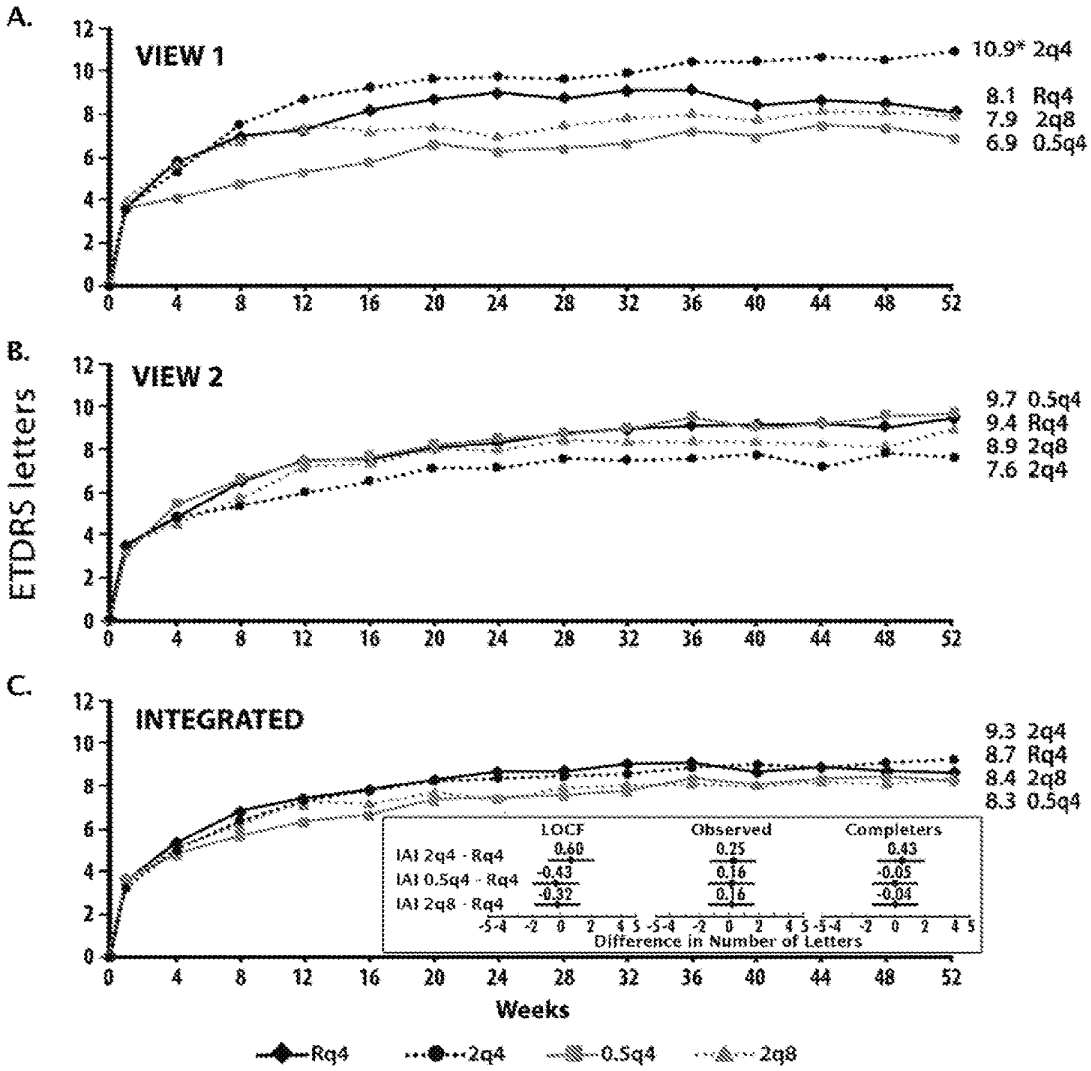


Figure 3. Mean change in best-corrected visual acuity (BCVA) from baseline to week 52 in the individual VIEW studies and in the integrated analysis. Values in the line graphs refer to mean changes in the number of letters from baseline at week 52. Only the intravitreal aflibercept 2q4 arm in VIEW 1 was significantly different from ranibizumab (*P = 0.005 for the difference). The panel inset (integrated analysis) shows the difference in visual acuity between each intravitreal aflibercept arm and ranibizumab (least-square mean with 95% confidence interval [CI]) at week 52, using 3 different analyses: by last observation carried forward (LOCF), using observed case data, and by assessing completers. Rq4 = 0.5 mg ranibizumab monthly; 0.5q4 = 0.5 mg IAI monthly; 2q4 = 2 mg IAI monthly; 2q8 = 2 mg IAI every 2 months after 3 initial monthly doses; ETDRS = Early Treatment Diabetic Retinopathy Study; IAI = intravitreal aflibercept injection.

Safety

Intravitreal aflibercept was generally well tolerated and had a profile of ocular treatment-emergent adverse experiences, including serious ocular adverse events, similar to those for monthly ranibizumab (Table 3; Appendix 7, available at http://aaojournal.org). Differences were noted in the prespecified analyses of intraocular pressure: Fewer patients treated with aflibercept had increases in intraocular pressure over the 52 weeks of the VIEW 1 and VIEW 2 studies (Appendix 7, available at http://aaojournal.org). There were few ocular injection–related treatment-emergent serious adverse events in the study eye. The combined data for both studies showed a rate of events/1000 injections of 1.1, 0.8, 0.1, and 0.2 for the ranibizumab 0.5q4 and intravitreal aflibercept 2q4, 0.5q4, and 2q8 groups, respectively. These events included eye disorders, endophthalmitis, procedural complications, and increased intraocular pressure.

There was a similar overall incidence of systemic (nonocular) adverse events (Appendix 7, available at http://aaojournal.org), serious systemic adverse events, specific arterial thromboembolic end points as set forth by the Anti-Platelet Trialists' Collaboration, and deaths between intravitreal aflibercept and ranibizumab (Table 3). Among the aflibercept treatment groups, there was no evidence of a dose-response for adverse events: The group with the highest exposure, the aflibercept 2q4 group, generally had the lowest rates of adverse events. There was little to no immunogenicity associated with intravitreal aflibercept (Appendix 8, available at http://aaojournal.org).

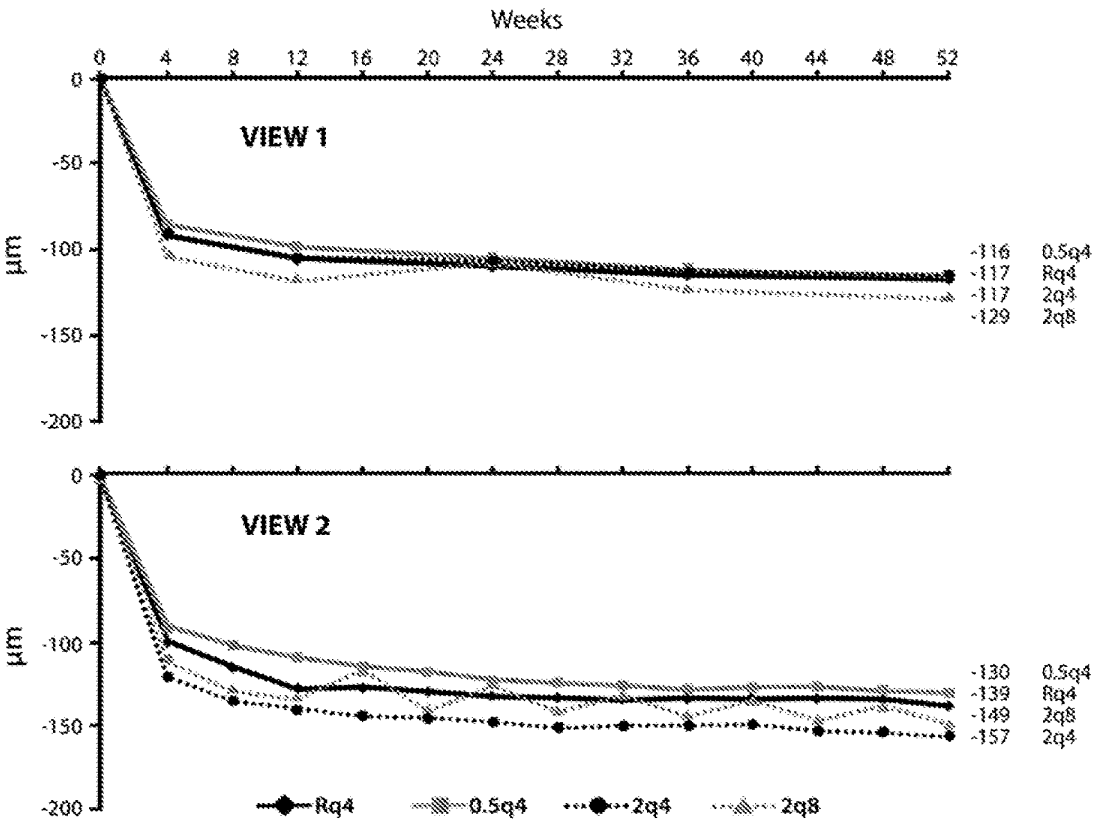


Figure 4. Mean change from baseline in central retinal thickness (full analysis set). As described in the "Materials and Methods" section, in VIEW 1, optical coherence tomography (OCT) was performed at screening, at the treatment initiation visit, and at weeks 4, 12, 24, 36, and 52 (and was optional at the investigators' discretion at other study visits). In VIEW 2, OCT was performed at every study visit. The last observation carried forward (LOCF) was used for imputing the missing values. Rq4 = 0.5 mg ranibizumab monthly; 0.5q4 = 0.5 mg intravitreal aflibercept injection (IAI) monthly; 2q4 = 2 mg IAI monthly; 2q8 = 2 mg IAI every 2 months after 3 initial monthly doses.

Discussion

We have described 2 large and similarly designed clinical trials involving more than 2400 patients with neovascular AMD. In both trials, all 3 aflibercept treatment regimens (including the every-2-month regimen after 3 initial monthly loading doses) were statistically noninferior to monthly ranibizumab in preventing moderate visual acuity loss at 1 year, meeting the primary outcome of the trials; all the aflibercept regimens also met the stricter margin of 5% for clinical equivalence compared with monthly ranibizumab. In terms of mean change in BCVA over time, all aflibercept regimens behaved similarly to monthly ranibizumab, with rapid increases after the first treatment followed by incremental gains that were durable and maintained through week 52. Mean visual acuity scores were within 1 letter of each other at week 52 in the prespecified integrated analysis combining the 2 studies; of note, aflibercept dosed every 2 months achieved a visual acuity score within 0.3 letters of monthly ranibizumab, with a CI of less than 2 letters, regardless of the analysis set used. Because the CATT[13] highlighted the inability of other regimens, including monthly bevacizumab and PRN ranibizumab or bevacizumab, to match the retinal thickness and retinal fluid improvements seen with monthly ranibizumab, it is notable that all 3 aflibercept regimens behaved similarly to monthly ranibizumab in terms of these anatomic measures.

Because of the large treatment burden, extensive efforts have been devoted toward developing an optimized treatment paradigm that avoids the need for monthly injections or monitoring visits. The CATT and HARBOR studies used noninferiority margins of change from baseline BCVA of 5 letters and 4 letters, respectively, to evaluate the efficacy of PRN regimens (Invest Ophthalmol Vis Sci 2012;53:E-Abstract 3677).[13] The CATT[13] generated much interest, in part because it showed that PRN ranibizumab and bevacizumab regimens approached the visual acuity outcomes achieved with monthly ranibizumab; however, these PRN regimens produced numerically smaller gains in BCVA at 52 weeks (by 1.7–2.6 letters) with poorer anatomic outcomes. Switching from a monthly to a PRN regimen during the second year of the CATT significantly worsened visual and anatomic out-

Table 3. Serious Ocular Adverse Events in the Study Eye and Other Key Nonocular Events Occurring in ≥0.5%* of Patients in Any Study Arm

	VIEW 1				VIEW 2			
	Ranibizumab	*Intravitreal Aflibercept*			*Ranibizumab*	*Intravitreal Aflibercept*		
	0.5q4	*2q4*	*0.5q4*	*2q8*	*0.5q4*	*2q4*	*0.5q4*	*2q8*
N (safety analysis set)	304	304	304	303	291	309	297	307
Patients with at least 1 ocular SAE, n (%)	10 (3.3)	7 (2.3)	6 (2.0)	3 (1.0)	9 (3.1)	6 (1.9)	5 (1.7)	9 (2.9)
Serious ocular adverse event, n (%)								
Endophthalmitis	3 (1.0)	3 (1.0)	0	0	0	0	0	0
Visual acuity reduced	2 (0.7)	1 (0.3)	2 (0.7)	0	1 (0.3)	1 (0.3)	1 (0.3)	5 (1.6)
Retinal hemorrhage	2 (0.7)	0	0	2 (0.7)	1 (0.3)	2 (0.6)	1 (0.3)	1 (0.3)
Posterior capsule opacification	—	—	—	—	2 (0.7)	0	0	0
Serious systemic (or nonocular) adverse event	57 (18.8)	40 (13.2)	50 (16.4)	51 (16.8)	26 (8.9)	36 (11.7)	37 (12.5)	38 (12.4)
APTC ATE events								
Any APTC ATE event	5 (1.6)	2 (0.7)	7 (2.3)	6 (2.0)	5 (1.7)	4 (1.3)	5 (1.7)	8 (2.6)
Vascular death	1 (0.3)	0	1 (0.3)	4 (1.3)	1 (0.3)	1 (0.3)	2 (0.7)	1 (0.3)
Nonfatal myocardial infarction	4 (1.3)	1 (0.3)	4 (1.3)	1 (0.3)	2 (0.7)	2 (0.6)	2 (0.7)	5 (1.6)
Nonfatal stroke	0	1 (0.3)	2 (0.7)	1 (0.3)	2 (0.7)	1 (0.3)	1 (0.3)	2 (0.7)
Any AE of hypertension	29 (9.5)	25 (8.2)	26 (8.6)	31 (10.2)	29 (10.0)	31 (10.0)	22 (7.4)	28 (9.1)
SAEs of interest occurring in any patient								
Venous thromboembolic event	1 (0.3%)	0	1 (0.3%)	0	0	0	0	0
Congestive heart failure event	2 (0.7%)	1 (0.3%)	2 (0.7%)	3 (1.0%)	1 (0.3%)	0	0	1 (0.3%)
GI perforation or fistula event	0	0	0	0	0	0	1 (0.3%)	1 (0.3%)
Nonocular hemorrhagic event	1 (0.3%)	1 (0.3%)	3 (1.0%)	3 (1.0%)	0	2 (0.6%)	0	1 (0.3%)
Delayed wound healing	0	0	0	0	0	0	0	0

0.5q4 = 0.5 mg monthly; 2q4 = 2 mg monthly; 2q8 = 2 mg every 2 months after 3 initial monthly doses; AE = adverse event; APTC ATE = Anti-platelet Trialists' Collaboration Arteriothrombolic Event; GI = gastrointestinal; SAE = serious adverse event.
*For SAEs of interest, occurrence in any patient is reported.

comes and resulted in a decrease in the proportion of patients without retinal fluid.[14] The results from the HARBOR study showed that PRN regimens of ranibizumab (including a higher 2 mg dose) did not achieve noninferiority compared with monthly ranibizumab (Invest Ophthalmol Vis Sci 2012;53:E-Abstract 3677). Moreover, the PRN regimens in both CATT and HARBOR still required mandatory monthly visits, during which treatment decisions had to be made largely on the basis of anatomic measures. The demonstration that monthly aflibercept provides similar efficacy and safety as the current approved standard of monthly ranibizumab is important, but the finding that remarkably similar improvement in vision and anatomic measures can be achieved with less than monthly intravitreal aflibercept injections and without requiring monthly monitoring visits provides an important advance for both patients and their treating physicians. The FDA has approved intravitreal aflibercept injection for AMD and recommended the regimen of 2 mg once every 2 months after 3 initial monthly doses (Eylea [package insert]. Tarrytown, NY: Regeneron Pharmaceuticals, Inc; 2011. Available at: http://www.regeneron.com/Eylea/eylea-fpi.pdf. Accessed August 8, 2012). This approval was based on the evaluation that this regimen provided the best benefit/risk; the approved label notes that aflibercept can be dosed as often as every 4 weeks, although additional efficacy was not reported with such frequent dosing. By halving the need for monthly visits, the every-2-month regimen of aflibercept may markedly decrease the treatment burden experienced by patients and their families. Less frequent injections also should provide an ocular safety benefit. Although the VIEW studies were not powered to see differences in rare but serious intraocular complications (e.g., endophthalmitis and retinal detachment), it is likely that fewer injections may substantially decrease the cumulative population risk of such events, considering that millions of injections are given each year.

After the 1-year primary end point of VIEW 1/VIEW 2 presented in this article, all treatment groups' dosing intervals were changed to a common protocol of modified quarterly dosing with their originally randomized dose and drug (all patients were monitored monthly and received a minimum of dosing every 12 weeks with interim as-needed monthly intravitreal injections). The results of this second year were recently presented (Invest Ophthalmol Vis Sci 2012;53:E-Abstract 6962) and reveal 81.6% to 85.7% patient retention in all groups with comparable visual acuity maintenance (91%–92%) in each group at the 96-week time point. The total number of active injections (baseline to week 96) was 16.0 to 16.2 in the monthly intravitreal aflibercept groups, 16.5 in the monthly ranibizumab group, and 11.2 in the original 2q8 group. The finding that visual acuity maintenance can be achieved for up to 96 weeks in the 2q8 group with similar gains in BCVA compared with ranibizumab despite more than 5 fewer doses is encouraging and implies that the treatment burden of neovascular AMD may be meaningfully reduced with this 2q8 intravitreal aflibercept regimen.

The sustained durability of intravitreal aflibercept as demonstrated by the every-2-month regimen is consistent

with the rationale that a higher binding affinity could lead to increased durability.[17] It is encouraging that the increased affinity of intravitreal aflibercept did not result in an observed increase in ocular or systemic adverse events. In the VIEW 1 and VIEW 2 trials, no differences in systemic or ocular safety were noted between any of the doses or dosing regimens of intravitreal aflibercept. Systemic exposure of aflibercept injected intravitreally is extremely low (Eylea [package insert]. Tarrytown, NY: Regeneron Pharmaceuticals, Inc; 2011. Available at: http://www.regeneron.com/Eylea/eylea-fpi.pdf. Accessed August 8, 2012). After intravitreal administration of 2 mg per eye of aflibercept to patients with wet AMD, the mean maximum concentration of free aflibercept in the plasma was 0.02 μg/ml (range, 0–0.054 μg/ml) and was attained in 1 to 3 days. The free aflibercept plasma concentrations were undetectable 2 weeks post-dosing in all patients. Aflibercept did not accumulate in plasma when administered as repeated doses intravitreally every 4 weeks. It is estimated that after intravitreal administration of 2 mg to patients, the mean maximum plasma concentration of free aflibercept is more than 100-fold lower than the concentration of aflibercept required to half-maximally bind systemic VEGF.

In conclusion, intravitreal aflibercept dosed monthly or every 2 months after 3 initial monthly doses resulted in similar visual and anatomic outcomes as ranibizumab dosed monthly, as well as similar safety and tolerability. Intravitreal aflibercept dosed every 2 months has the potential to provide patients, their families, and clinicians the opportunity for the optimal vision gains and anatomic disease control they have come to expect from monthly ranibizumab, with a substantially decreased treatment and compliance burden, and a lower cumulative risk of injection-related adverse events.

Acknowledgments. The authors thank Karen Chu, MS, Regeneron Pharmaceuticals, Inc, and Avner Ingerman, MD, initial study director of VIEW 1 and former employee of Regeneron Pharmaceuticals, Inc, for assistance with the design of the studies. Editorial and administrative assistance to the authors who wrote the manuscript was provided by S. Balachandra Dass, PhD, Regeneron Pharmaceuticals, Inc.

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Footnotes and Financial Disclosures

Originally received: July 26, 2012.
Final revision: August 30, 2012.
Accepted: September 4, 2012.
Available online: October 17, 2012. Manuscript no. 2012-1120.

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Financial Disclosure(s):
The author(s) have made the following disclosure(s): J.S.H. is a consultant to and has received research funding from Alimera, Allergan, Fovea, Genentech, Genzyme, GlaxoSmithKline, Neovista, and Regeneron Pharmaceuticals. He has also received travel support from Regeneron Pharmaceuticals. D.M.B. is a consultant to Alimera, Allergan, Bayer, Genentech/Roche, Novartis, Regeneron Pharmaceuticals, and Thrombogenics and has received research funding from Alcon, Alimera, Allergan, Eli Lilly, Genentech, GlaxoSmithKline, Novartis, Regeneron Pharmaceuticals, and Thrombogenics. He has also received travel support from Regeneron Pharmaceuticals and lecture fees from Genentech. V.C. is a consultant to Alimera and Bayer and has received research funding from Alcon, Allergan, Bayer, Novartis, and Pfizer. He is an advisory board member for Allergan and Novartis and has also received travel support from Bayer. J.-F.K. is a consultant to Alcon, Bayer, and Thea and an advisory board member for Allergan, Bayer, and Novartis. He has received travel support from Regeneron Pharmaceuticals. P.K.K. is a consultant to Bayer, Genentech, Novartis, and Regeneron Pharmaceuticals. He has received research funding from Regeneron Pharmaceuticals. Q.D.N. is a consultant to Bausch & Lomb and Santen and has received research funding from Genentech, Novartis, and Pfizer. B.K. has received travel support from Bayer. A.H. is a consultant to Alcon, Allergan, Centocor, Johnson & Johnson, Neovista, Merck, Ophthotech, Oraya, Paloma, P.R.N., Q.L.T., Regeneron Pharmaceuticals, and Thrombogenics. He has received research funding and lecture fees from Alcon, Allergan, Genentech, Neovista, Ophthotech, Oraya, P.R.N., Q.L.T., Regeneron Pharmaceuticals, and Second Sight. Y.O. is a consultant to Alcon and Bayer and has received travel support from Bayer. G.D.Y., N.S., R.V., A.J.B., and Y.S. are employees of Regeneron Pharmaceuticals. MA, G.G., B.S., and R.S. are employees of Bayer HealthCare. C.S.'s institution has received payments from the Medical University of Vienna for data monitoring/reviewing and statistical analysis. U.S.-E. is a consultant to Alcon, Allergan, Bayer HealthCare, and Novartis, and an advisory board member for Alcon and Novartis. She has received travel support from Bayer HealthCare and lecture fees from Bayer HealthCare and Novartis.

G.D.Y. and N.S., incorporating the advice of a panel of academic and physician experts, developed the initial proposal for the VIEW 1 study design. The study design of both studies was further developed and finalized by the academic authors and clinical and statistical authors from Regeneron Pharmaceuticals and Bayer HealthCare (sponsors). The sponsors conducted the trials and together with the investigators gathered the data. Study conduct and analyses were supervised by the Study Steering Committees and the sponsors. The Writing Committee consisting of authors J.S.H., D.M.B., V.C., and U.S.-E. (subteam of VIEW Steering Committees) along with G.D.Y. composed the first draft of the paper, which was critically revised and finalized by the input of all coauthors. The Writing Committee members and all other authors met authorship criteria. All coauthors had full and unrestricted access to the data and decided to publish the paper vouching for the accuracy and completeness of the reported data.

Funding: Sponsored by Regeneron Pharmaceuticals, Inc, Tarrytown, New York, and Bayer HealthCare, Berlin Germany. The sponsors participated in the design and conduct of the study, analysis of the data, and preparation of the manuscript.

Presented at: the American Academy of Ophthalmology Annual Meeting, October 22–25, 2011, Orlando, Florida. This is an Annual poster presentation.

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Electronic Patent Application Fee Transmittal

Application Number:	13940370
Filing Date:	12-Jul-2013
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Filer:	Karl Bozicevic/Kimberly Zuehlke
Attorney Docket Number:	REGN-008CIP (725A1-US)

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Claims in Excess of 20	1202	6	80	480
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**480**

Electronic Acknowledgement Receipt	
EFS ID:	23464679
Application Number:	13940370
International Application Number:	
Confirmation Number:	1055
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-008CIP (725A1-US)
Receipt Date:	11-SEP-2015
Filing Date:	12-JUL-2013
Time Stamp:	13:41:36
Application Type:	Utility under 35 USC 111(a)

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	Amendment/Req. Reconsideration-After Non-Final Reject		1	1	
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/940,370	Filing Date 07/12/2013	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 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).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	09/11/2015	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 26	Minus	** 20	= 6	X $80 =	480
	Independent (37 CFR 1.16(h))	* 2	Minus	***3	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**480**

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
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96387 7590 10/19/2015

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EXAMINER	
LOCKARD, JON MCCLELLAND	
ART UNIT	PAPER NUMBER
1647	

DATE MAILED: 10/19/2015

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/940,370	07/12/2013	George D. YANCOPOULOS	REGN-008CIP (725A1-US)	1055

TITLE OF INVENTION: USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	01/19/2016

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.

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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.

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96387 7590 10/19/2015

Regeneron
Bozicevic, Field & Francis
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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.

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/940,370	07/12/2013	George D. YANCOPOULOS	REGN-008CIP (725A1-US)	1055

TITLE OF INVENTION: USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	01/19/2016

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 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

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 _____

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❑ 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 credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

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

❑ Applicant certifying micro entity status. See 37 CFR 1.29

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/940,370	07/12/2013	George D. YANCOPOULOS	REGN-008CIP (725A1-US)	1055

EXAMINER
LOCKARD, JON MCCLELLAND

ART UNIT	PAPER NUMBER
1647	

DATE MAILED: 10/19/2015

96387 7590 10/19/2015

Regeneron
Bozicevic, Field & Francis
1900 University Ave
Suite 200
East Palo Alto, CA 94303

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

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

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.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B 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. 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.

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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.
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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.
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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. 13/940,370	Applicant(s) YANCOPOULOS, GEORGE D.	
	Examiner JON M. LOCKARD	Art Unit 1647	AIA (First Inventor to File) Status No

-- 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 Amendment filed 11 September 2015*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
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 *1-7,12-14 and 18-33 (renumbered as claims 1-17,19,20,18 and 21-26, respectively)*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   Certified copies:
   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. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ 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).
6. ☐ 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. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☐ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

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

Inventor Information for 13/940370

/J.L./

Inventor Name	City	State/Country
YANCOPOULOS, GEORGE D.	YORKTOWN HEIGHTS	NEW YORK

Appln Info | Contents | Petition Info | Atty/Agent Info | Continuity Data | Foreign Data | **Inventors** | Applicants | Address | Fees | Post Info | Pre Gra

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13940370	YANCOPOULOS, GEORGE D.
	Examiner	Art Unit
	JON M LOCKARD	1647

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
NONE		6/13/2015	JML

SEARCH NOTES		
Search Notes	Date	Examiner
STIC Search of SEQ ID NOs:1-2. See sequence search results in SCORE.	6/13/2015	JML
EAST (USPAT, US-PGPUB, EPO, DERWENT): See attached search history.	6/13/2015	JML
STN (MEDLINE, SCISEARCH, EMBASE, BIOSIS): See attached search history.	6/13/2015	JML
PALM: Inventor search.	6/13/2015	JML

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
	STIC Search of SEQ ID NOs:1-2. See sequence search results in SCORE.	9/29/2015	JML
	EAST (USPAT, UPAD): See attached search history.	9/29/2015	JML
	PALM: Inventor search.	9/29/2015	JML

EAST Search History

EAST Search History (Interference) /J.L./

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1493	(flt1 or vegfr1 or (vegf adj r1)) same ((flk1 or kdr or vegfr2 or (vegf adj r2)) or (Flt4 vegfr3 or (vegf adj r3)))	USPAT; UPAD	OR	ON	2015/09/29 09:37
L2	380	l1 and ((chimer$ or fusion) same vegf)	USPAT; UPAD	OR	ON	2015/09/29 09:37
L3	1470	(flt1 or vegfr1 or (vegf adj r1)) with ((flk1 or kdr or vegfr2 or (vegf adj r2)) or (Flt4 vegfr3 or (vegf adj r3)))	USPAT; UPAD	OR	ON	2015/09/29 09:38
L4	375	l3 and ((chimer$ or fusion) same vegf)	USPAT; UPAD	OR	ON	2015/09/29 09:38
L5	114	l1 same ((chimer$ or fusion) same vegf)	USPAT; UPAD	OR	ON	2015/09/29 09:38
L6	110	l3 same ((chimer$ or fusion) same vegf)	USPAT; UPAD	OR	ON	2015/09/29 09:38
L7	57	l3 with ((chimer$ or fusion) with vegf)	USPAT; UPAD	OR	ON	2015/09/29 09:38
L8	55	(l5 or l6) and ((eye or ocular or retina$ or macular) with disorder)	USPAT; UPAD	OR	ON	2015/09/29 09:39
L9	3	(l5 or l6) same ((eye or ocular or retina$ or macular) with disorder)	USPAT; UPAD	OR	ON	2015/09/29 09:39
L10	30	l7 and (((eye or ocular) with disorder) or (macular adj degeneration) or (diabetic adj retinopathy))	USPAT; UPAD	OR	ON	2015/09/29 09:39
L11	3	l7 with (((eye or ocular) with disorder) or (macular adj degeneration) or (diabetic adj retinopathy))	USPAT; UPAD	OR	ON	2015/09/29 09:39
L12	108	yancopoulos-g$.in.	USPAT; UPAD	OR	ON	2015/09/29 09:40
L13	17	l8 and l12	USPAT; UPAD	OR	ON	2015/09/29 09:40

9/29/2015 9:40:40 AM
C:\Users\jlockard\Documents\EAST\Workspaces\13940370.wsp

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BIB DATA SHEET

CONFIRMATION NO. 1055

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
13/940,370	07/12/2013	424	1647	REGN-008CIP (725A1-US)

APPLICANTS
REGENERON PHARMACEUTICASS, INC., Tarrytown, NY;

INVENTORS
George D. YANCOPOULOS, Yorktown Heights, NY;

**** CONTINUING DATA ***************************
This application is a CIP of PCT/US2012/020855 01/11/2012
which claims benefit of 61/432,245 01/13/2011
and claims benefit of 61/434,836 01/21/2011
and claims benefit of 61/561,957 11/21/2011

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****
07/29/2013

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☐ No Verified and Acknowledged /JON MCCLELLAND LOCKARD/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		NY	1	20	1

ADDRESS
Regeneron
Bozicevic, Field & Francis
1900 University Ave
Suite 200
East Palo Alto, CA 94303
UNITED STATES

TITLE
USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

FILING FEE RECEIVED	FEES: Authority has been given in Paper	☐ All Fees
2360	No._____ to charge/credit DEPOSIT ACCOUNT	☐ 1.16 Fees (Filing)
	No._____ for following:	☐ 1.17 Fees (Processing Ext. of time)
		☐ 1.18 Fees (Issue)
		☐ Other _____
		☐ Credit

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13940370	YANCOPOULOS, GEORGE D.
	Examiner	**Art Unit**
	JON M LOCKARD	1647

CPC							
Symbol						**Type**	**Version**
A61K		47	/	48415		F	2013-01-01
A61K		38	/	179		I	2013-01-01
C07K		16	/	22		I	2013-01-01
C07K		14	/	71		I	2013-01-01
A61K		2039	/	505		A	2013-01-01
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CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

/JON M LOCKARD/ Examiner.Art Unit 1647	09/29/2015	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	26	
/CHRISTINE J SAOUD/ Primary Examiner.Art Unit 1647	09/29/2015	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13940370	YANCOPOULOS, GEORGE D.
	Examiner	**Art Unit**
	JON M LOCKARD	1647

US ORIGINAL CLASSIFICATION		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				

/JON M LOCKARD/ Examiner.Art Unit 1647 (Assistant Examiner)	09/29/2015 (Date)	**Total Claims Allowed:** 26	
/CHRISTINE J SAOUD/ Primary Examiner.Art Unit 1647 (Primary Examiner)	09/29/2015 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13940370	YANCOPOULOS, GEORGE D.
	Examiner	**Art Unit**
	JON M LOCKARD	1647

☐ 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	1	17	24												
2	2	19	25												
3	3	20	26												
4	4	18	27												
5	5	21	28												
6	6	22	29												
7	7	23	30												
8	12	24	31												
9	13	25	32												
10	14	26	33												
11	18														
12	19														
13	20														
14	21														
15	22														
16	23														

/JON M LOCKARD/ Examiner.Art Unit 1647 (Assistant Examiner)	09/29/2015 (Date)	**Total Claims Allowed:** 26	
/CHRISTINE J SAOUD/ Primary Examiner.Art Unit 1647 (Primary Examiner)	09/29/2015 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure NONE

Electronically Filed

AMENDMENT UNDER 37 C.F.R. §1.312 Address to: Mail Stop: Issue Fee P.O. Box 1450 Alexandria, VA 22313-1450	Attorney Docket No.	REGN-008CIP
	Confirmation No.	1055
	First Named Inventor	YANCOPOULOS, GEORGE D.
	Application Number	13/940,370
	Filing Date	July 12, 2013
	Group Art Unit	1647
	Examiner Name	LOCKARD, JON MCCLELLAND
	Title: "Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders"	

Sir:

This amendment is further to the Notice of Allowance dated October 19, 2015. Applicants request formal amendments to claims.

The claims begin on page 2 of this document.

The remarks begin on page 5 of this document.

In view of the amendments to the claims, and remarks put forth below, reconsideration and allowance are respectfully requested.

AMENDMENTS TO THE CLAIMS

1. (**Currently Amended**) A method for treating an angiogenic eye disorder in a patient, said method comprising sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist;

wherein each secondary dose is administered 2 to 4 weeks after the immediately preceding dose; and

wherein each tertiary dose is administered at least 8 weeks after the immediately preceding dose**;**

wherein the **VEGF antagonist is a** VEGF receptor-based chimeric molecule ~~**comprises**~~ **comprising** (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130-231 of SEQ ID NO:2; and (3) a multimerization component comprising amino acids 232-457 of SEQ ID NO:2.

2. (Original) The method of claim 1, wherein only a single secondary dose is administered to the patient, and wherein the single secondary dose is administered 4 weeks after the initial dose of the VEGF antagonist.

3. (Original) The method of claim 1, wherein only two secondary doses are administered to the patient, and wherein each secondary dose is administered 4 weeks after the immediately preceding dose.

4. (Original) The method of claim 3, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

5. (Original) The method of claim 1, wherein at least 5 tertiary doses of the VEGF antagonist are administered to the patient, and wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered 8 or 12 weeks after the immediately preceding dose.

6. (Original) The method of claim 1, wherein the angiogenic eye disorder is selected from the group consisting of: age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization.

7. (Original) The method of claim 6, wherein the angiogenic eye disorder is age related macular degeneration.

8.-11. (**Canceled**)

12. (Original) The method of claim 1, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

13. (Original) The method of claim 12, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

14. (Original) The method of claim 13, wherein the intraocular administration is intravitreal administration.

15. – 17. (**Canceled**)

18. (**Previously Presented**) The method of claim 14, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

19. (Original) The method of claim 18, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

20. (Original) The method of claim 18, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

21. (**Currently Amended**) A method for treating an angiogenic eye disorder in a patient, said method comprising sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist;

wherein each secondary dose is administered 2 to 4 weeks after the immediately preceding dose; and

wherein each tertiary dose is administered at least 8 weeks after the immediately preceding dose;

wherein the **VEGF antagonist is a** VEGF receptor-based chimeric molecule ~~**comprises**~~ **comprising** VEGFR1R2-FcΔC1(a) encoded by the nucleic acid sequence of SEQ ID NO:1.

22. (**Previously Presented**) The method of claim 21, wherein only a single secondary dose is administered to the patient, and wherein the single secondary dose is administered 4 weeks after the initial dose of the VEGF antagonist.

23. (**Previously Presented**) The method of claim 21, wherein only two secondary doses are administered to the patient, and wherein each secondary dose is administered 4 weeks after the immediately preceding dose.

24. (**Previously Presented**) The method of claim 23, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

25. (**Previously Presented**) The method of claim 21, wherein at least 5 tertiary doses of the VEGF antagonist are administered to the patient, and wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered 8 or 12 weeks after the immediately preceding dose.

26. (**Previously Presented**) The method of claim 21, wherein the angiogenic eye disorder is selected from the group consisting of: age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization.

27. (**Previously Presented**) The method of claim 24, wherein the angiogenic eye disorder is age related macular degeneration.

28. (**Previously Presented**) The method of claim 21, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

29. (**Previously Presented**) The method of claim 28, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

30. (**Previously Presented**) The method of claim 29, wherein the intraocular administration is intravitreal administration.

31. (**Previously Presented**) The method of claim 30, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

32. (**Previously Presented**) The method of claim 31, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

33. (**Previously Presented**) The method of claim 31, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

REMARKS

FORMAL MATTERS

Claims 1-7, and 12-14 and 18-33 are now pending in this application.

Claims 1 and 21 have been amended.

The proposed amendments to claims 1 and 21 are formal in nature and made in order to ensure that both claims have a proper basis for the term "VEGF receptor-based chimeric molecule."

No new matter has been added.

REQUESTED AMENDMENTS

Applicants recognize the Examiner has considerable discretion with respect to the entry of amendments after a Notice of Allowance. However, in this situation, the claim amendment does not, in any way, change the substance of the claims. Further, the amendment does further clarify the basis for a claim term. Accordingly, the claim amendment is respectfully requested.

CONCLUSION

The requested amendment would clarify the claims and not add new matter or require further search and/or consideration on part of the Examiner. Thus, entry of the amendment is requested.

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-008CIP.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 23 October 2015

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
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Facsimile: (650) 327-3231

Electronic Acknowledgement Receipt

EFS ID:	23867487
Application Number:	13940370
International Application Number:	
Confirmation Number:	1055
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-008CIP (725A1-US)
Receipt Date:	23-OCT-2015
Filing Date:	12-JUL-2013
Time Stamp:	13:43:25
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		REGN-008CIP_10-23-2015_312_amend.pdf	47561 934555ff69a17bc0aa5ea4abcb48813ef71e4e03	yes	5

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment after Notice of Allowance (Rule 312)	1	1
	Claims	2	4
	Applicant Arguments/Remarks Made in an Amendment	5	5
Warnings:			
Information:			
	Total Files Size (in bytes):	47561	

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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.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/940,370	07/12/2013	George D. YANCOPOULOS	REGN-008CIP (725A1-US)	1055

96387 7590 11/04/2015

Regeneron
Bozicevic, Field & Francis
1900 University Ave
Suite 200
East Palo Alto, CA 94303

EXAMINER	
LOCKARD, JON MCCLELLAND	

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
11/04/2015	ELECTRONIC

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.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docket@bozpat.com

Response to Rule 312 Communication	Application No.	Applicant(s)
	13/940,370	YANCOPOULOS, GEORGE D.
	Examiner	Art Unit
	JON M. LOCKARD	1647

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

1. ☒ The amendment filed on *23 October 2015* under 37 CFR 1.312 has been considered, and has been:

a) ☐ entered.

b) ☒ entered as directed to matters of form not affecting the scope of the invention.

c) ☐ disapproved because the amendment was filed after the payment of the issue fee.
Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.

d) ☐ disapproved. See explanation below.

e) ☐ entered in part. See explanation below.

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

OK TO ENTER: /J.L./
10/28/2015

Electronically Filed

AMENDMENT UNDER 37 C.F.R. §1.312 Address to: Mail Stop: Issue Fee P.O. Box 1450 Alexandria, VA 22313-1450	Attorney Docket No.	REGN-008CIP
	Confirmation No.	1055
	First Named Inventor	YANCOPOULOS, GEORGE D.
	Application Number	13/940,370
	Filing Date	July 12, 2013
	Group Art Unit	1647
	Examiner Name	LOCKARD, JON MCCLELLAND
	Title: "Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders"	

Sir:

This amendment is further to the Notice of Allowance dated October 19, 2015. Applicants request formal amendments to claims.

The claims begin on page 2 of this document.

The remarks begin on page 5 of this document.

In view of the amendments to the claims, and remarks put forth below, reconsideration and allowance are respectfully requested.

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13940370
	Filing Date	2013-07-12
	First Named Inventor	George D. YANCOPOULOS
	Art Unit	1647
	Examiner Name	LOCKARD, Jon McClelland
	Attorney Docket Number	REGN-008CIP

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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
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	1	2010-509369	JP		2010-03-25	Genentech, Inc.	See WO 2008/063932 for English Equivalent	☒
	2	2008/063932	WO		2008-05-29	Genentech, Inc.		☐

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	Application Number	13940370
	Filing Date	2013-07-12
	First Named Inventor	George D. YANCOPOULOS
	Art Unit	1647
	Examiner Name	LOCKARD, Jon McClelland
	Attorney Docket Number	REGN-008CIP

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	NGUYEN et al., "A phase I trial of an IV-administered vascular endothelial growth factor trap for treatment in patients with choroidal neovascularization due to age-related macular degeneration" Ophthalmology (Sept 2006) 113 (9):1522e1-1522e14 (epub July 28,2006)	☐

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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	13940370
	Filing Date	2013-07-12
	First Named Inventor	George D. YANCOPOULOS
	Art Unit	1647
	Examiner Name	LOCKARD, Jon McClelland
	Attorney Docket Number	REGN-008CIP

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.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

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	/Karl Bozicevic, Reg. No. 28,807/	Date (YYYY-MM-DD)	2015-11-19
Name/Print	Karl Bozicevic	Registration Number	28807

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.**

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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.

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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 inspections 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.

(19)日本国特許庁(JP)　(12)公表特許公報(A)　(11)特許出願公表番号
特表2010-509369
(P2010-509369A)
(43)公表日　平成22年3月25日(2010.3.25)

(51)Int.Cl.		F I		テーマコード（参考）
A61K 45/00	(2006.01)	A61K 45/00		4C084
A61K 39/395	(2006.01)	A61K 39/395	N	4C085
A61P 27/02	(2006.01)	A61K 39/395	V	
A61P 9/10	(2006.01)	A61P 27/02		
		A61P 9/10		

審査請求 未請求　予備審査請求 未請求　（全 33 頁）

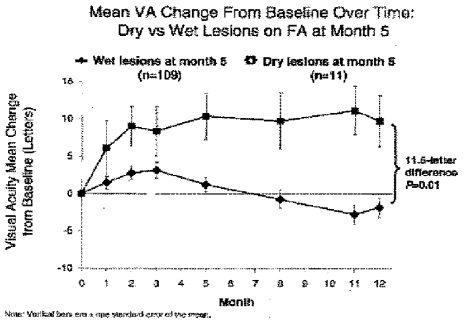
(21)出願番号　特願2009-536520 (P2009-536520)
(86)(22)出願日　平成19年11月9日 (2007.11.9)
(85)翻訳文提出日　平成21年7月1日 (2009.7.1)
(86)国際出願番号　PCT/US2007/084320
(87)国際公開番号　WO2008/063932
(87)国際公開日　平成20年5月29日 (2008.5.29)
(31)優先権主張番号　60/865,380
(32)優先日　平成18年11月10日 (2006.11.10)
(33)優先権主張国　米国 (US)

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最終頁に続く

(54)【発明の名称】加齢黄斑変性症を処置するための方法

(57)【要約】
加齢黄斑変性症（ＡＭＤ）は、高齢者の間での重症な不可逆性の視力喪失の主要な原因である。本発明は、ＶＥＧＦアンタゴニストで加齢黄斑変性症を処置する方法に関する。一部の実施形態では、ＶＥＧＦアンタゴニストは、抗ＶＥＧＦ抗体である。一部の実施形態では、抗ＶＥＧＦ抗体は全長抗ＶＥＧＦ抗体であり、他の実施形態では、抗ＶＥＧＦ抗体は抗体フラグメントである。一部の実施形態では、抗体フラグメントは、ラニビズマブを含むＦａｂ抗体フラグメントである。様々な治療化合物の有効性を増大させる、新規投与法は、ＡＭＤの処置に役立つ。加齢黄斑変性症を患っているか、またはそのリスクがある哺乳動物に、投与する方法を提供する。



【特許請求の範囲】
【請求項1】
以前にＶＥＧＦアンタゴニスト治療を受けたことのある患者の眼において浸潤型加齢黄斑変性症（ＡＭＤ）を処置する方法であって、前記患者にＶＥＧＦアンタゴニストの投薬を投与することを含み、前記眼の平均中心窩の厚さが正常値を超えず、および前記投薬が前回のＶＥＧＦアンタゴニスト治療から１ヵ月以上後に投与される、方法。
【請求項2】
前記投薬が前回のＶＥＧＦアンタゴニスト治療から３ヵ月後に投与される、請求項１に記載の方法。
【請求項3】
前記患者の前記平均中心窩の厚さが２２５μｍを超えない、請求項１に記載の方法。
【請求項4】
哺乳動物の前記平均中心窩の厚さが２００μｍを超えない、請求項３に記載の方法。
【請求項5】
前記眼の前記平均中心窩の厚さの測定値を得ることをさらに含む、請求項１に記載の方法。
【請求項6】
前記ＶＥＧＦアンタゴニストが抗ＶＥＧＦ抗体である、請求項1に記載の方法。
【請求項7】
前記抗ＶＥＧＦ抗体が全長抗ＶＥＧＦ抗体である、請求項６に記載の方法。
【請求項8】
前記抗ＶＥＧＦ抗体が抗体フラグメントである、請求項６に記載の方法。
【請求項9】
前記抗体フラグメントがＦａｂ抗体フラグメントである、請求項８に記載の方法。
【請求項10】
前記Ｆａｂ抗体フラグメントがラニビズマブである、請求項９に記載の方法。
【請求項11】
患者の眼における浸潤型ＡＭＤを処置する方法であって、
（ａ）前記患者に、ＶＥＧＦアンタゴニストの第１の投薬を投与することと、
（ｂ）患眼の前記平均中心窩の厚さの測定値を得ることと、
（ｃ）前記平均中心窩の厚みが正常値以上である場合、前記ＶＥＧＦアンタゴニストの第２の投薬を前記患者に投与することと
を含む、方法。
【請求項12】
前記平均中心窩の厚さが少なくとも２５０μｍである、請求項１１に記載の方法。
【請求項13】
前記平均中心窩の厚さが少なくとも２７５μｍである、請求項１２に記載の方法。

【発明の詳細な説明】
【技術分野】
【0001】
（関連する出願への相互参照）
本願は、米国特許法のもとでなされた特許出願であり、２００６年１１月１０日に出願された米国仮特許出願第６０／８６５，３８０号に対する米国特許法のもとでの優先権を主張する。米国仮特許出願第６０／８６５，３８０号の内容は、本明細書中に参考として援用される。
【0002】
本発明は、ＶＥＧＦアンタゴニストで加齢黄斑変性症を処置する方法に関する。
【背景技術】
【0003】
血管新生は、眼内新生血管疾患（例えば増殖性網膜症、加齢黄斑変性症（ＡＭＤ））、

ならびに他の種々の疾患の発症に結びつけられる。この１０年間にわたり行われた研究は、正常な血管新生および異常な血管新生の制御における血管内皮増殖因子（ＶＥＧＦ）の重要な役割を確立した（非特許文献１）。さらに、ＶＥＧＦが腫瘍および眼内障害に付随する新血管新生の重要なメディエーターであることが示されている（非特許文献１）。

【0004】

加齢黄斑変性症（ＡＭＤ）は、高齢者の間での重症な不可逆性の視力喪失の主要な原因である。非特許文献２。ＡＭＤは、広範囲の臨床所見および病理所見（例えばドルーゼとして公知の淡黄色の斑、網膜色素上皮（ＲＰＥ）の崩壊、脈絡膜新生血管（ＣＮＶ）、および円板状黄斑変性症）によって特徴づけられる。この疾患の徴候は、２つの型に分類される。すなわち、非滲出型（乾燥型）および滲出型（浸潤型または新生血管型）

ＡＭＤにおける新血管新生は、中心窩下脈絡膜新生血管病変の蛍光眼底血管造影法に基づいて様々なパターンに分類されることができる。非特許文献３。主要な血管造影パターンは、典型的パターンおよび潜在的パターンと称され、かつ攻撃性の様々な程度、視力喪失、および異なる治療法の選択肢への応答に関連する。

【図面の簡単な説明】

【0013】

【図1】実施例１の研究を模式的に表す。

【図2】ＶＥＧＦアンタゴニストを用いる、例えば加齢黄斑変性症（ＡＭＤ）を処置するための投与計画を模式的に表す。

【図3】実施例１に記載する検査の過程にわたる患者の視力を示す。

【図4】実施例１に記載する１２ヵ月目の検査において、ベースライン視力から１５文字未満を喪失した患者の比率を示す。

【図5】実施例１に記載する１２ヵ月目の検査において、ベースライン視力から少なくとも１５文字を回復した患者の比率を示す。

【図6】実施例１に記載する検査において、２ヵ月目の中心窩網膜の厚さに基づき細分した、２集団の患者の視力を示す。

【図7】実施例１に記載する検査において、３ヵ月目の中心窩網膜の厚さに基づき細分した、２集団の患者の視力を示す。

【図8】実施例１に記載する検査において、５ヵ月目の中心窩網膜の厚さに基づき細分した、２集団の患者の視力を示す。

【図9】実施例１に記載する検査において、５ヵ月目の中心窩網膜の厚さに基づき細分した、ベースライン視力から１５文字未満を喪失した患者の１２カ月目の比率を示す。

【図10】実施例１に記載する検査において、５ヵ月目の中心窩網膜の厚さに基づき細分した、ベースライン視力から少なくとも１５文字を回復した患者の１２カ月目の比率を示す。

【図11】実施例１に記載する検査において、３ヵ月目で浸潤型病変または乾燥型病変を有するかどうかに基づき細分した患者の視力を示す。

【図12】実施例１に記載する検査において、５ヵ月目で浸潤型病変または乾燥型病変を有するかどうかに基づき細分した患者の視力を示す。

【図13】実施例１に記載する検査において、５ヵ月目で浸潤型病変または乾燥型病変を有するかどうかに基づき細分した、ベースライン視力から１５文字未満を喪失した患者の１２カ月目の比率を示す。

【図14】実施例１に記載する検査において、５ヵ月目で浸潤型病変または乾燥型病変を有するかどうかに基づき細分した、ベースライン視力から少なくとも１５文字を回復した患者の１２カ月目の比率を示す。

【図1】

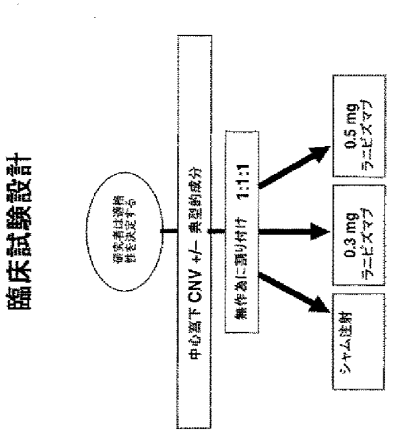

臨床試験設計
中心窩下 CNV +/− 典型的成分
無作為に割り付け 1:1:1
シャム注射
0.3 mg ラニビズマブ
0.5 mg ラニビズマブ

Figure 1

【図2】

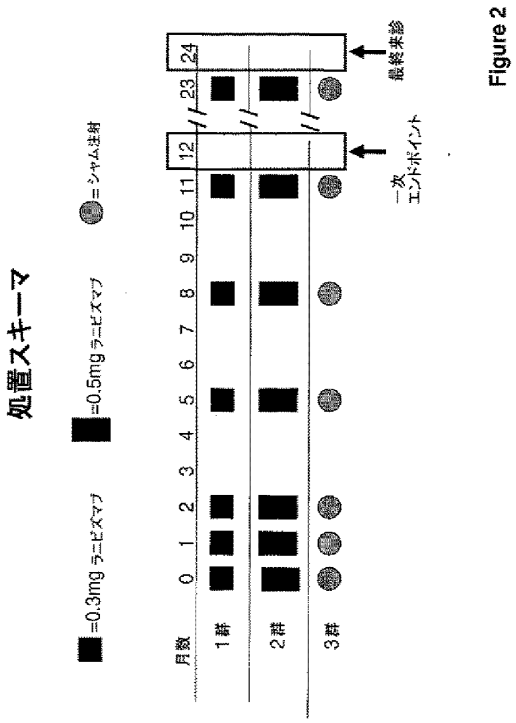

処置スキーマ
=0.3mg ラニビズマブ
=0.5mg ラニビズマブ
=シャム注射
月数
0 1 2 3 4 5 6 7 8 9 10 11 12 23 24
1群
2群
3群
一次エンドポイント
最終来診

Figure 2

【図3】

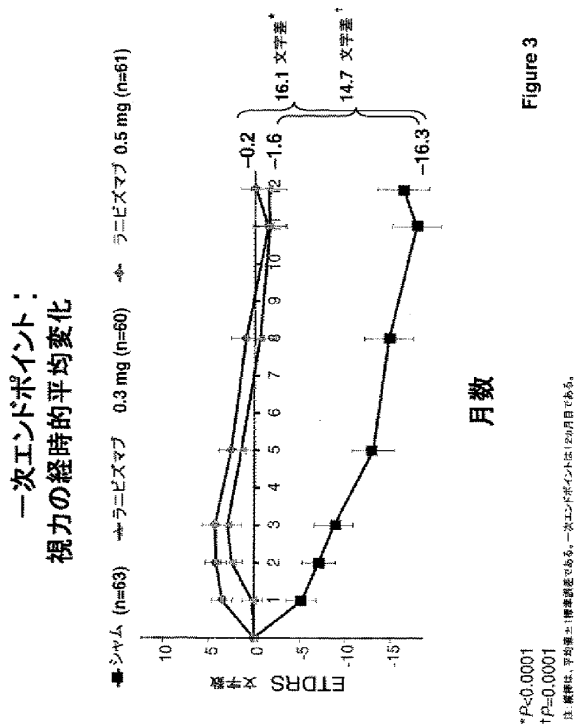

一次エンドポイント：
視力の経時的平均変化
シャム (n=63)
ラニビズマブ 0.3 mg (n=60)
ラニビズマブ 0.5 mg (n=61)
ETDRS 文字数
月数
-0.2
-1.6
-16.3
16.1 文字差*
14.7 文字差†
*P<0.0001
†P=0.0001

Figure 3

【図4】

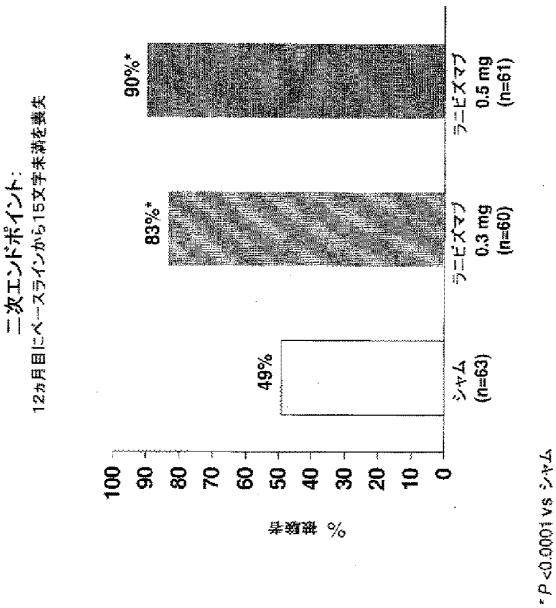

二次エンドポイント:
12カ月目にベースラインから15文字未満を喪失
% 被験者
100
90
80
70
60
50
40
30
20
10
0
49%
83%*
90%*
シャム (n=63)
ラニビズマブ 0.3 mg (n=60)
ラニビズマブ 0.5 mg (n=61)
*P <0.0001 vs シャム

Figure 4

【図5】

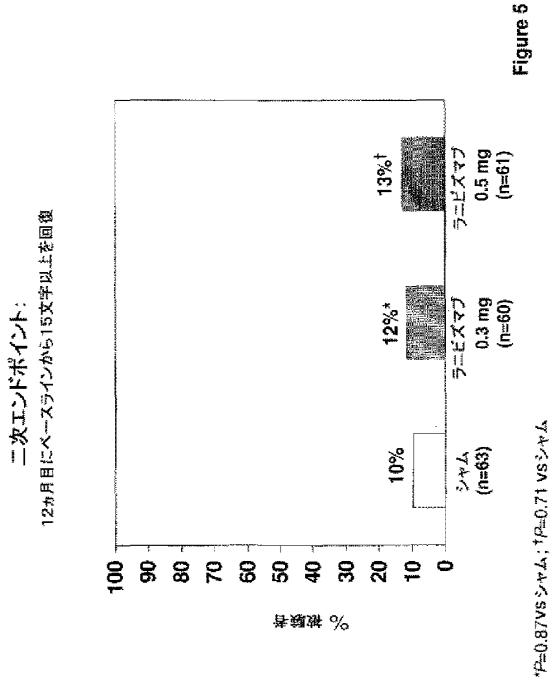

二次エンドポイント:
12カ月にベースラインから15文字以上を回復
% 被験者
100
90
80
70
60
50
40
30
20
10
0
10%
12%*
13%†
シャム
(n=63)
ラニビズマブ
0.3 mg
(n=60)
ラニビズマブ
0.5 mg
(n=61)
*P=0.87vsシャム; †P=0.71 vsシャム

Figure 5

【図6】

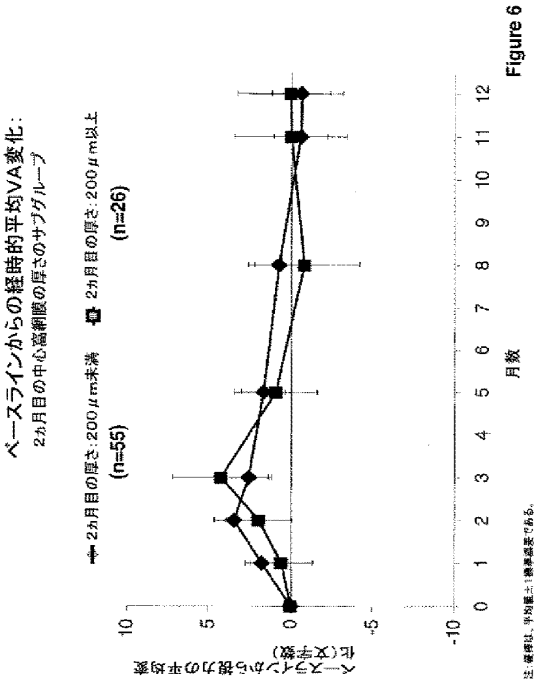

ベースラインからの経時的平均VA変化：
2カ月目の中心窩網膜の厚さのサブグループ
2カ月目の厚さ：200μm未満 (n=55)
2カ月目の厚さ：200μm以上 (n=26)
ベースラインからの視力の平均変化（文字数）
10
5
0
-5
-10
0 1 2 3 4 5 6 7 8 9 10 11 12
月数

Figure 6

【図7】

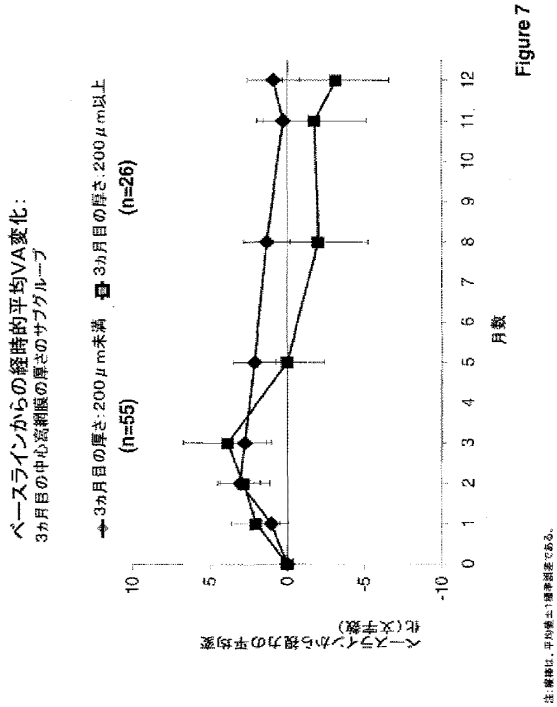


Figure 7

注：棒線は、平均値±1標準誤差である。

【図8】

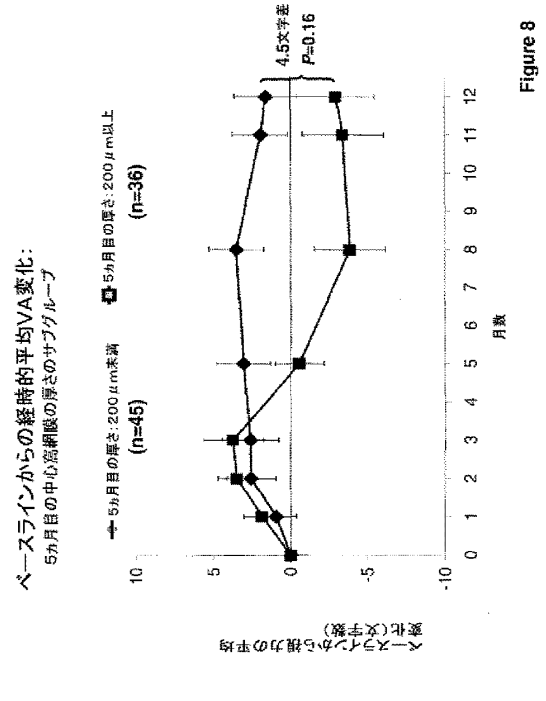

ベースラインからの経時的平均VA変化：
5カ月目の中心窩網膜の厚さのサブグループ
5カ月目の厚さ：200μm未満 (n=45)
5カ月目の厚さ：200μm以上 (n=36)
4.5文字差
P=0.16
ベースラインからの視力の平均変化（文字数）
月数
10
5
0
-5
-10
0 1 2 3 4 5 6 7 8 9 10 11 12
注：棒線は、平均値±1標準誤差である。

Figure 8

【図9】

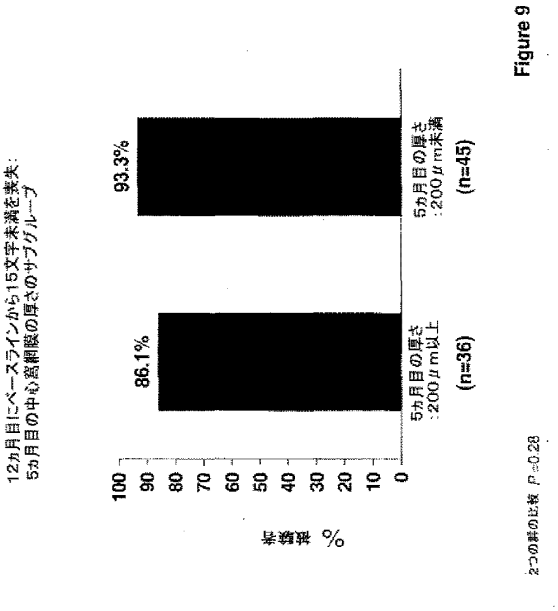

12カ月目にベースラインから15文字未満を喪失：
5カ月目の中心窩網膜の厚さのサブグループ
93.3%
86.1%
100
90
80
70
60
50
40
30
20
10
0
患者数 %
5カ月目の厚さ
:200μm以上
(n=36)
5カ月目の厚さ
:200μm未満
(n=45)
2つの群の比較 P=0.28

Figure 9

【図10】

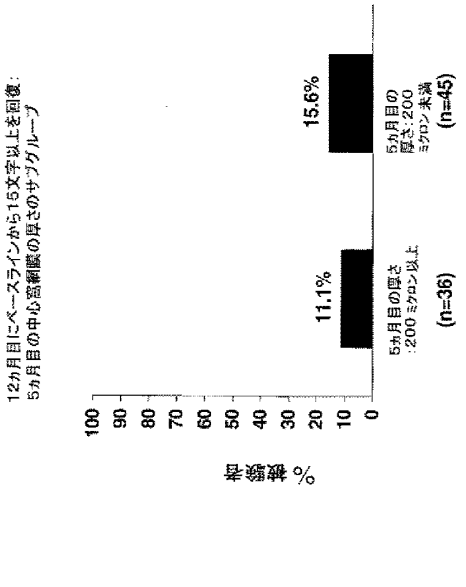


Figure 10

【図11】

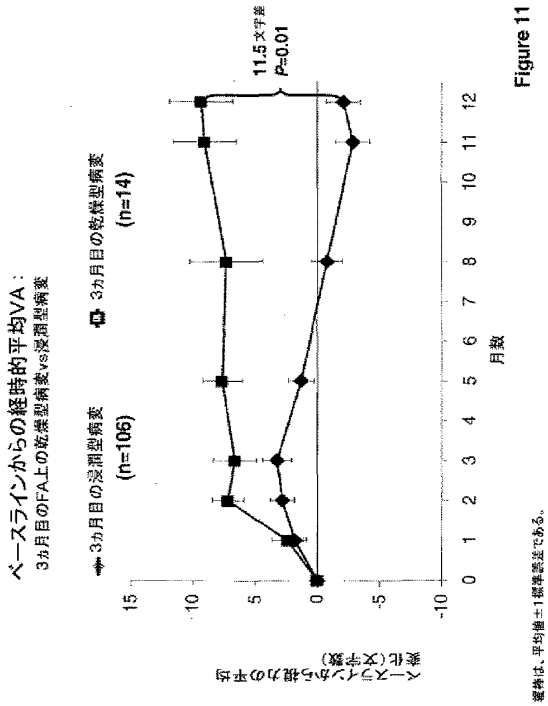


Figure 11

【図12】

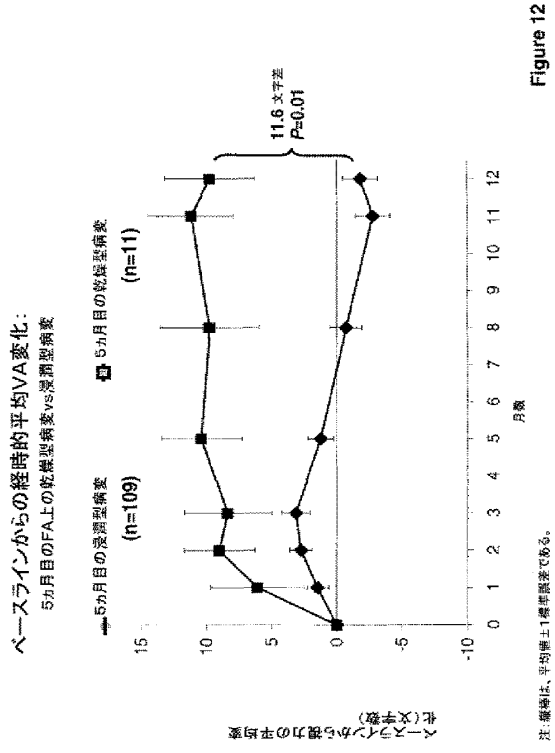


Figure 12

【図13】

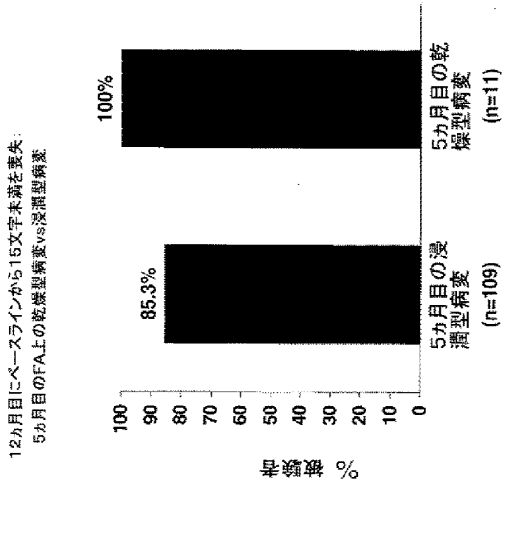


Figure 13

【図14】

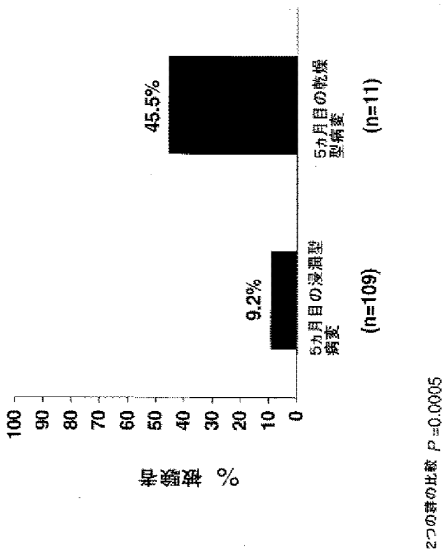


Figure 14

【国際調査報告】

【図14】

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/084320

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395

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, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HEIER J S ET AL: "Long-term experience with lucentis (ranibizumab) in patients with neovascular age-related macular degeneration (AMD)" IOVS, vol. 46, no. Suppl. S, 2005, page 1393, XP009099781 & ANNUAL MEETING OF THE ASSOCIATION-FOR-RESEARCH-IN-VISION-AND-OPHTHALMOLOGY; FT LAUDERDALE, FL, USA; MAY 01 -05, 2005 ISSN: 0146-0404 Results abstract -/--	1-13

[X] Further documents are listed in the continuation of Box C.　　[ ] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed.
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Date of the actual completion of the international search	Date of mailing of the international search report
30 May 2008	10/07/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Didelon, Frédéric

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/084320

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"Results From A Phase IIIb Study Showed Patients With Wet AMD Treated With Lucentis Quarterly Experienced A 16-Letter Benefit Over The Controls" INTERNET CITATION, [Online] 3 June 2006 (2006-06-03), XP002414531 Retrieved from the Internet: URL:http://www.medicalnewstoday.com/printerfriendlynews.php?newsid=44460> [retrieved on 2007-01-10] the whole document	1-13
X	ANONYMOUS: "Lucentis- Ranibizumab injection (Highlights of prescribing information)" INTERNET ARTICLE, [Online] 16 October 2006 (2006-10-16), XP002481626 Retrieved from the Internet: URL:http://web.archive.org/web/20061016124130/http://www.gene.com/gene/products/information/pdf/lucentis-prescribing.pdf> [retrieved on 2008-05-06] Dosing abstract	1-13
X	ROSENFELD P: "An Update on Bevacizumab" REVIEW OF OPHTALMOLOGY, [Online] vol. 12, no. 12, 12 January 2005 (2005-01-12), XP002414532 Retrieved from the Internet: URL:http://www.revophth.com/index.asp?page=1_857.htm> [retrieved on 2007-01-10] paragraph [0002] - paragraph [0003]	1-9
X	GRAGOUDAS E S ET AL: "PEGAPTANIB FOR NEOVASCULAR AGE-RELATED MACULAR DEGENERATION" NEW ENGLAND JOURNAL OF MEDICINE, THE, MASSACHUSETTS MEDICAL SOCIETY, WALTHAM, MA, US, vol. 351, 1 January 2004 (2004-01-01), pages 2805-2816, XP008064513 ISSN: 0028-4793 abstract	1-5
A	JERRY HELZNER: "Inside genenthech's Lucentis Strategy" RETINAL PHYSICIAN, [Online] September 2005 (2005-09), XP002481627 Retrieved from the Internet: URL:http://www.retinalphysician.com/article.aspx?article=100112> [retrieved on 2008-05-06] page 2, last paragraph page 4	1-13

-/--

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/084320

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	THE PIER STUDY GROUP REGILLO ET AL: "Randomized, Double-Masked, Sham-Controlled Trial of Ranibizumab for Neovascular Age-related Macular Degeneration: PIER Study Year 1" AMERICAN JOURNAL OF OPHTHALMOLOGY, OPHTHALMIC PUBL., CHICAGO, IL, US, vol. 145, no. 2, 25 January 2008 (2008-01-25), pages 239-248.e5, XP022433903 ISSN: 0002-9394 abstract	1-13
P,X	SPAIDE ET AL: "Ranibizumab According to Need: A Treatment for Age-related Macular Degeneration" AMERICAN JOURNAL OF OPHTHALMOLOGY, OPHTHALMIC PUBL., CHICAGO, IL, US, vol. 143, no. 4, 24 March 2007 (2007-03-24), pages 679-680, XP022000736 ISSN: 0002-9394 the whole document	1-13

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(81)指定国　　AP(BW,GH,GM,KE,LS,MW,MZ,NA,SD,SL,SZ,TZ,UG,ZM,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,BG,CH,CY,CZ,DE,DK,EE,ES,FI,FR,GB,GR,HU,IE,IS,IT,LT,LU,LV,MC,MT,NL,PL,PT,RO,SE,SI,SK,TR),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GQ,GW,ML,MR,NE,SN,TD,TG),AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BH,BR,BW,BY,BZ,CA,CH,CN,CO,CR,CU,CZ,DE,DK,DM,DO,DZ,EC,EE,EG,ES,FI,GB,GD,GE,GH,GM,GT,HN,HR,HU,ID,IL,IN,IS,JP,KE,KG,KM,KN,KP,KR,KZ,LA,LC,LK,LR,LS,LT,LU,LY,MA,MD,ME,MG,MK,MN,MW,MX,MY,MZ,NA,NG,NI,NO,NZ,OM,PG,PH,PL,PT,RO,RS,RU,SC,SD,SE,SG,SK,SL,SM,SV,SY,TJ,TM,TN,TR,TT,TZ,UA,UG,US,UZ,VC,VN,ZA,ZM,ZW

Ｆターム(参考)　4C084 AA17　DC50　NA14　ZA331 ZA361
　　4C085 AA14　AA16　BB41　BB43　CC23

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 May 2008 (29.05.2008)

PCT

(10) International Publication Number
WO 2008/063932 A2

(51) International Patent Classification:
C07K 16/00 (2006.01)

(21) International Application Number:
PCT/US2007/084320

(22) International Filing Date:
9 November 2007 (09.11.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/865,380 10 November 2006 (10.11.2006) US

(71) Applicant *(for all designated States except US)*: **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, California 94080 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **SHAMS, Naveed** [US/US]; 216 Dove Creek Lane, Danville, California 94506 (US).

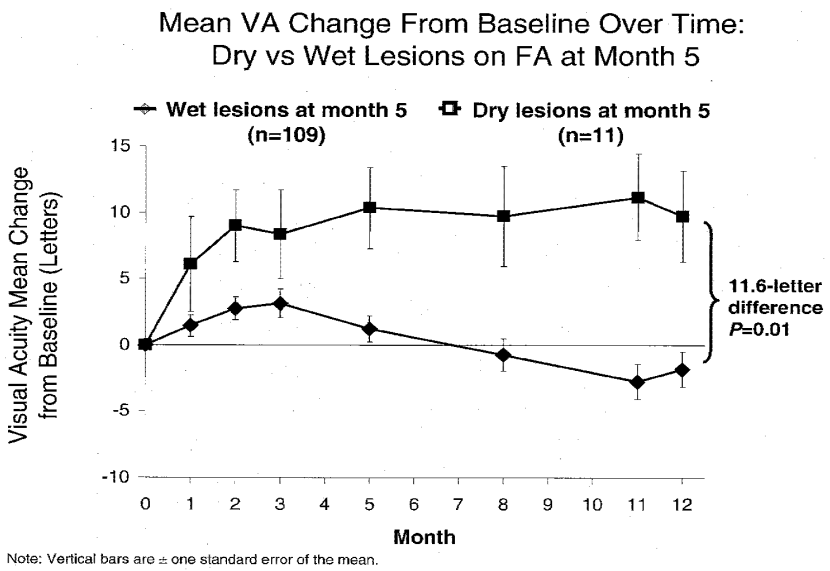
(74) Agents: **KALINOWSKI, Grant** et al.; 1 DNA Way, South San Francisco, California 94080 (US).

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



(57) **Abstract:** A method is provided for administering to a mammal suffering from, or at risk for, age-related macular degeneration.

WO 2008/063932 A2

METHOD FOR TREATING AGE-RELATED MACULAR DEGENERATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a non-provisional application filed under 37 CFR 1.53(b)(1), claiming priority under 35 USC 119(e) to provisional application number 60/865,380 filed November 10, 2006, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] This invention relates to methods for treating age-related macular degeneration with a VEGF antagonist.

BACKGROUND OF THE INVENTION

[0003] 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. Work done over the last decade 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)). Furthermore, VEGF has been shown to be a key mediator of neovascularization associated with tumors and intraocular disorders (Ferrara *et al.*).

[0004] 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).

[0005] The neovascularization in AMD can be classified into different patterns based on fluorescein angiography of subfoveal chorodial 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.

[0006] Anti-VEGF neutralizing antibodies inhibit intraocular angiogenesis in models of ischemic retinal disorders (Adamis *et al. Arch. Ophthalmol.* 114:66-71 (1996)). Recently, an anti-VEGF antibody fragment, ranibizumab (LUCENTIS®), was approved for use in treatment of AMD. However, new methods of administering this and other therapeutic compounds, which increases their effectiveness would be valuable in the treatment of AMD.

SUMMARY OF THE INVENTION

[0007] The invention is based in part on the discovery that a particular population of age-related macular degeneration patients benefits from less frequent dosing with a VEGF antagonist. 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.

[0008] In one aspect, the invention provides a method for treating wet form age-related macular degeneration (AMD) in an eye of a patient who has had previous VEGF antagonist therapy, comprising administering to the patient a dose of a VEGF antagonist, wherein the mean foveal thickness of the eye is not more than normal and wherein the dose is administered more than one month after the previous VEGF antagonist therapy. In some embodiments, the dose is administered three months after the previous VEGF antagonist therapy. In some embodiments, the mean foveal thickness of the patient is not more than 225 μm or not more than 200 μm. In some embodiment, the method also comprises obtaining a measurement of the mean foveal thickness of the eye.

[0009] In some embodiments, the VEGF antagonist is an anti-VEGF antibody. In some embodiments, the anti-VEGF antibody is a full length anti-VEGF antibody and in others the it is an antibody fragment. In some embodiments the antibody fragment is a Fab antibody fragment, including ranibizumab.

[0010] In one aspect, the invention provides a method for treating wet form age-related macular degeneration (AMD) in an eye of a patient who has had previous VEGF antagonist therapy, comprising administering to the patient a dose of a VEGF antagonist, wherein the lesions of the eye are dry lesions and wherein the dose is administered more than one month after the previous VEGF antagonist therapy.

[0011] In one aspect, the invention provides a method for treating wet form AMD in an eye of a patient comprising: administering to the patient a first dose of a VEGF antagonist; obtaining a measurement of the mean foveal thickness of an affected eye; and administering to the patient a second dose of the VEGF antagonist if the mean foveal thickness is more than

normal. In some embodiments, the mean foveal thickness is at least 250 μm or at least 275 μm. In some embodiments, the first does is not administered.

[0012] 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.

BRIEF DESCRIPTION OF THE FIGURES

[0013] Figure 1 schematically illustrates the study in Example 1.

[0014] Figure 2 schematically illustrates a dosing regimen for treating, e.g., age-related macular degeneration (AMD) with a VEGF antagonist.

[0015] Figure 3 shows visual acuity of patients over the course of the study described in Example 1.

[0016] Figure 4 shows the proportion of patients in the study described in Example 1 at month 12 who had lost fewer than 15 letters from their baseline vision.

[0017] Figure 5 shows the proportion of patients in the study described in Example 1 at month 12 who had gained at least 15 letters from their baseline vision.

[0018] Figure 6 shows visual acuity of two populations of patients in the study described in Example 1 subdivided based on foveal retinal thickness at month 2.

[0019] Figure 7 shows visual acuity of two populations of patients in the study described in Example 1 subdivided based on foveal retinal thickness at month 3.

[0020] Figure 8 shows visual acuity of two populations of patients in the study described in Example 1 subdivided based on foveal retinal thickness at month 5.

[0021] Figure 9 shows the proportion of patients in the study described in Example 1 at month 12 who had lost fewer than 15 letters from their baseline vision subdivided based on foveal retinal thickness at month 5.

[0022] Figure 10 shows the proportion of patients in the study described in Example 1 at month 12 who had gained at least 15 letters from their baseline vision subdivided based on foveal retinal thickness at month 5.

[0023] Figure 11 shows visual acuity of patients in the study described in Example 1 subdivided based on whether they had wet lesion or dry lesions at month 3.

[0024] Figure 12 shows visual acuity of patients in the study described in Example 1 subdivided based on whether they had wet lesion or dry lesions at month 5.

[0025] Figure 13 shows the proportion of patients in the study described in Example 1 at month 12 who had lost fewer than 15 letters from their baseline vision subdivided based on whether they had wet lesion or dry lesions at month 5.

[0026] Figure 14 shows the proportion of patients in the study described in Example 1 at month 12 who had gained at least 15 letters from their baseline vision subdivided based on whether they had wet lesion or dry lesions at month 5.

DETAILED DESCRIPTION

Definitions

[0027] 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.

[0028] 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.

[0029] 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), $VEGF_{121}$-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 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, 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 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).

[0030] 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 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 effector function or may not be capable of binding complement or participating in ADCC.

[0031] 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*-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.

[0032] 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.

[0033] "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.

[0034] "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.

[0035] 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.

[0036] 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.

[0037] "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.

[0038] 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).

[0039] 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.

[0040] 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.

[0041] "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.

[0042] 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.

[0043] 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.

[0044] 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.

[0045] "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 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 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., *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).

[0046] 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 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 in Clackson *et al., Nature* 352:624-628 (1991) and Marks *et al., J. Mol. Biol.* 222:581-597 (1991), for example.

[0047] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or 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; and Morrison *et al., Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)).

[0048] "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 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 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 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).

[0049] 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.

[0050] 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.

[0051] By "Fc region chain" herein is meant one of the two polypeptide chains of an Fc region.

[0052] 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. 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.

[0053] 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 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.

[0054] "Hinge region" is generally defined as stretching from about Glu216, or about Cys226, to 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 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.

[0055] A "functional Fc region" possesses at least one "effector function" of a native sequence Fc 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.

[0056] A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature.

[0057] 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.

[0058] "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 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).

[0059] "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.

[0060] 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)).

[0061] "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.

[0062] 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).

[0063] 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-gly-gly-ser.

[0064] "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 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, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

[0065] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which 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., Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993).

[0066] 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 antigen binding regions. Linear antibodies can be bispecific or monospecific.

[0067] 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.

[0068] 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.

[0069] 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.

[0070] 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.,* IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

[0071] 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).

[0072] 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, e.g., antibodies to VEGF, antibodies to VEGF receptors, small molecules that block VEGF receptor signaling (e.g., PTK787/ZK2284, SU6668). Anti-angiogensis 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 & 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).

[0073] 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 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 fluorescein angiography, etc.

[0074] 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.

[0075] 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.

[0076] 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.

[0077] 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.

[0078] 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

[0079] The present invention describes a method for treatment of AMD which is based on analyses of affected eye(s), e.g. mean foveal thickness before or during a course of treatment or whether lesions in the affected eye(s) are wet lesions or dry lesions. The methods of the present invention allow one to predictably decrease subsequent doses of the therapeutic compound, while at the same time maintaining the therapeutic efficacy.

[0080] 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.

[0081] 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.

[0082] 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. 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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.

[0088] 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 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.

[0089] 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.

[0090] 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)).

[0091] Studies have documented the immunohistochemical localization of VEGF in surgically resected CNV membranes from AMD patients. Kvanta et al. (1996) demonstrated the presence of VEGF mRNA and protein in RPE cells and fibroblast like cells. *See* Kvanta 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).

[0092] An increase in VEGF expression has been noted in experimental models of CNV in rats and in non human primates (Husain et al., *Ophthalmology* 104:124250 (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 neovacularization reminiscent of CNV seen in humans with neovascular AMD. This further supports the involvement of VEGF in ocular neovascularization.

[0093] 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)).

[0094] 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.

[0095] Thus, VEGF antagonists are good therapeutic compounds for treating intraocular neovascular diseases.

[0096] 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 peptides compounds can be prepared by known solid-phase synthesis or recombinant DNA technology which are well known in the art.

[0097] 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.

[0098] 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.

[0099] 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

[0100] Therapeutic compounds 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 dextrins; 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 TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0101] 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-(methylmethacylate) 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).

[0102] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[0103] 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

[0104] 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

[0105] 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.

[0106] 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.

[0107] An example of a VEGF antagonist is ranibizumab (LUCENTIS®). 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.

[0108] *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.

[0109] 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.

[0110] 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.

[0111] A 30 gauge, ½-inch needle attached to a low volume (e.g., tuberculin) syringe containing 50 μL 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 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.

[0112] *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 injection, the injecting physician(s) performs the same post-injection procedures as those performed on subjects receiving ranibizumab.

[0113] *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 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.

[0114] • The supplies are assembled and 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.

[0115] • 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).

[0116] • 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.

[0117] • A sterile ophthalmic drape can be placed to isolate the field, and the speculum can be placed underneath the eyelid of the study eye.

[0118] • 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.

[0119] • Wait 90 seconds.

[0120] • 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).

[0121] • 1% lidocaine (without epinephrine) is injected subconjunctivally.

[0122] • A sterile 4X4 pad in a single wipe can be used to absorb excess liquid and to dry the periocular skin.

[0123] • The subject is instructed to direct gaze away from syringe prior to ranibizumab or sham injection.

[0124] *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.

[0125] After preparing the study eye as outlined above, 0.2 mL ranibizumab dose solution is withdrawn through a 5-μm 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.

[0126] 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 (±10 minutes) following the ranibizumab injection.

[0127] *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).

[0128] *Preparation and Administration of the Sham Injection*: See above for detailed instructions for pre-injection procedures.

[0129] 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.

[0130] 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.

[0131] 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.

[0132] 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 Raninizumab, 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.

[0133] *Measuring Macular Thickness in Patients during Therapy.* Mean foveal thickness of patients is measured using optical coherence tomography. Mean foveal thickness is ordinarily around 200 μm (see, e.g., Chan et al., Arch. Ophthalmol. 124:193-98 (2006)), but in individuals with age-related macular degeneration tends to be greater than average.

[0134] The overall results of this study are shown in Figures 3-5.

Example 2: Dose Frequency and Predicting Therapeutic Outcome

[0135] Mean foveal thickness in effected eyes of patients in the study was measured at various times during the first year of treatment. We compared the mean foveal thickness to the visual

acuity measurement at month 12. We found that patients who had mean foveal thickness that was not more than normal at months 3 and 5 maintained the initial improvement from the initial three injections whereas patients who had mean foveal thickness that was greater than normal at months 3 and 5 did not maintain this initial improvement (Figures 6-10). Thus, mean foveal thickness after initial VEGF antagonist therapy was predictive of visual acuity outcome at month 12.

[0136] In addition, lesions in effected eyes were analyzed by fundus photography and fluorescein angiography and lesions were scored as either "wet lesions" or "dry lesions". These data also were compared to the visual acuity measurement at month 12. We found that patients whose lesions were dry at months 3 and 5 maintained the initial improvement from the initial three injections whereas patients whose lesions were wet at months 3 and 5 did not maintain this initial improvement (Figures 11-14). Thus, dry lesions after initial VEGF antagonist therapy was predictive of visual acuity outcome at month 12.

[0137] 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 (AMD) in an eye of a patient who has had previous VEGF antagonist therapy, comprising administering to the patient a dose of a VEGF antagonist, wherein the mean foveal thickness of the eye is not more than normal and wherein the dose is administered more than one month after the previous VEGF antagonist therapy.

2. The method of claim 1, wherein the dose is administered three months after the previous VEGF antagonist therapy.

3. The method of claim 1, wherein the mean foveal thickness of the patient is not more than 225 μm.

4. The method of claim 3, wherein the mean foveal thickness of the mammal is not more than 200 μm.

5. The method of claim 1, further comprising obtaining a measurement of the mean foveal thickness of the eye.

6. The method of claim 1, wherein the VEGF antagonist is an anti-VEGF antibody.

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.

9. The method of claim 8, the antibody fragment is a Fab antibody fragment.

10. The method of claim 9, wherein the Fab antibody fragment is ranibizumab.

11. A method for treating wet form AMD in an eye of a patient comprising:

(a) administering to the patient a first dose of a VEGF antagonist;

(b) obtaining a measurement of the mean foveal thickness of an affected eye; and

(c) administering to the patient a second dose of the VEGF antagonist if the mean foveal thickness is more than normal.

12. The method of claim 11, wherein the mean foveal thickness is at least 250 μm.

13. The method of claim 12, wherein when the mean foveal thickness is at least 275 μm.

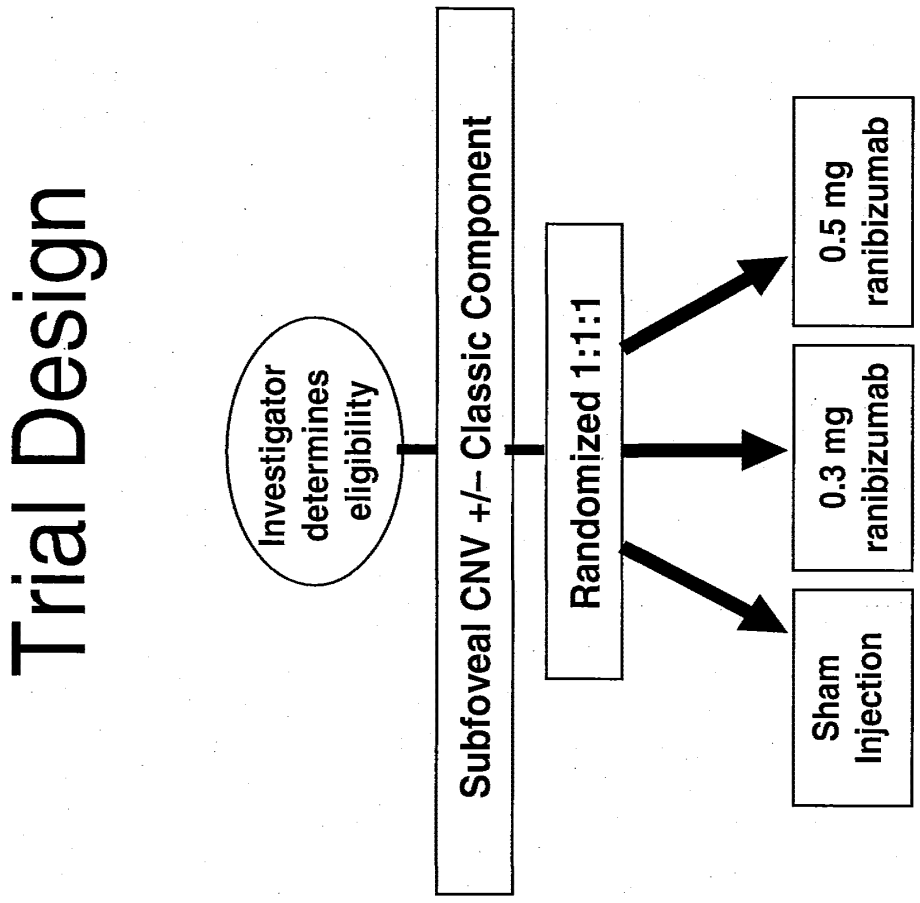

Trial Design
Investigator determines eligibility
Subfoveal CNV +/– Classic Component
Randomized 1:1:1
Sham Injection
0.3 mg ranibizumab
0.5 mg ranibizumab

Figure 1

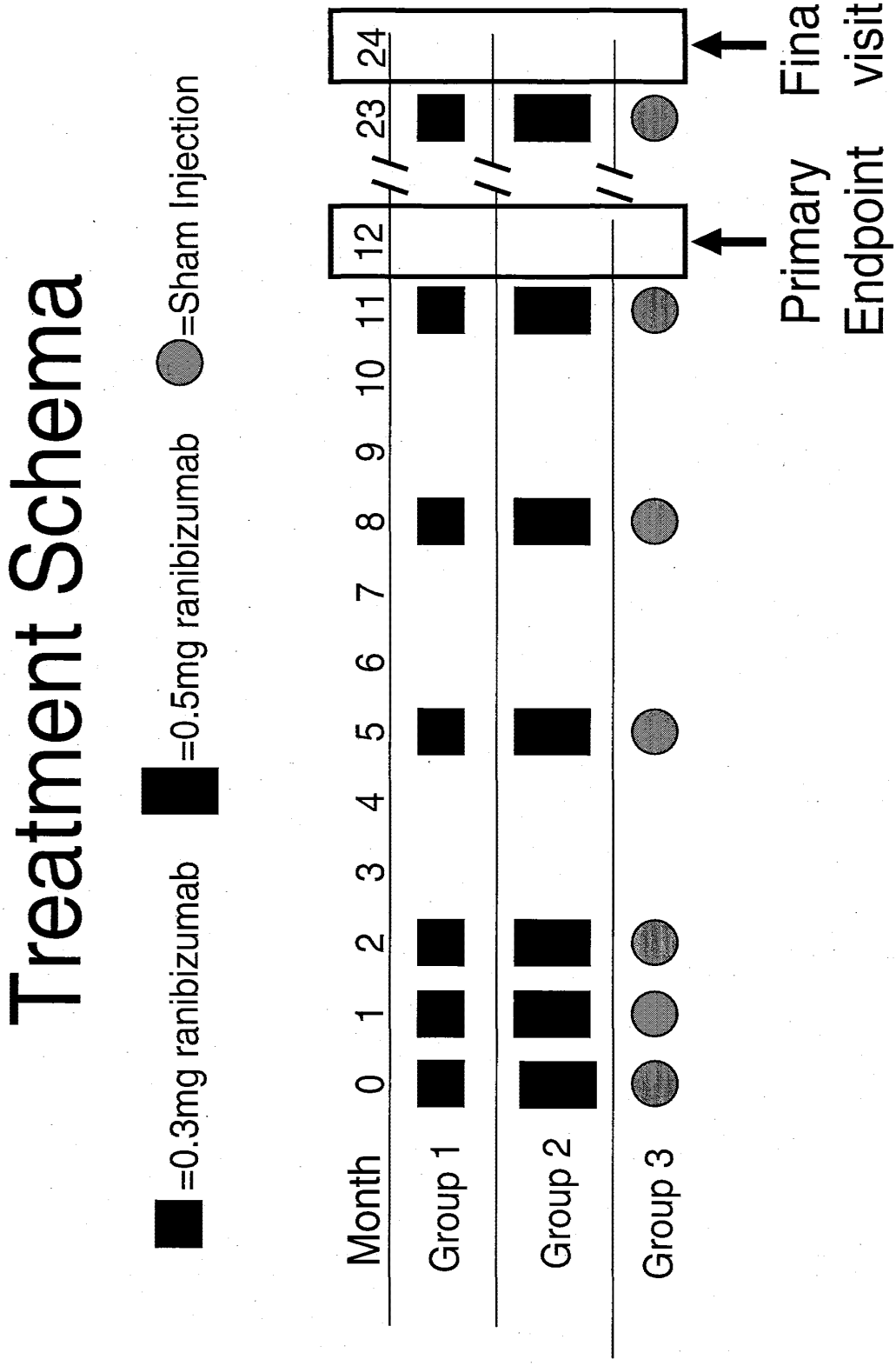

Treatment Schema
=0.3mg ranibizumab
=0.5mg ranibizumab
=Sham Injection
Month 0 1 2 3 4 5 6 7 8 9 10 11 12 23 24
Group 1
Group 2
Group 3
Primary Endpoint
Final visit

Figure 2

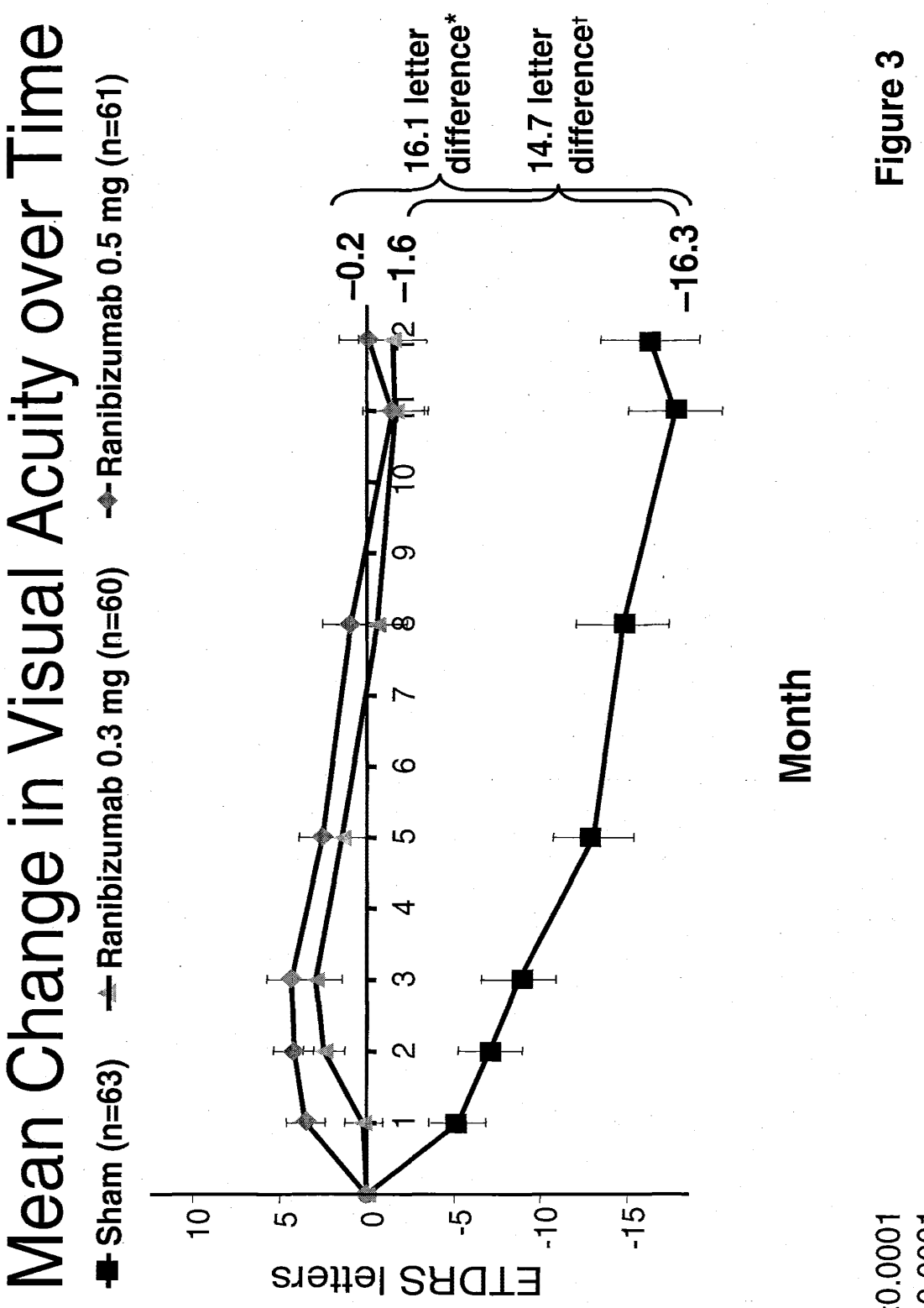


Figure 3

*P<0.0001
†P=0.0001

Note: Vertical bars are ± one standard error of the mean. The primary endpoint is at 12 months.

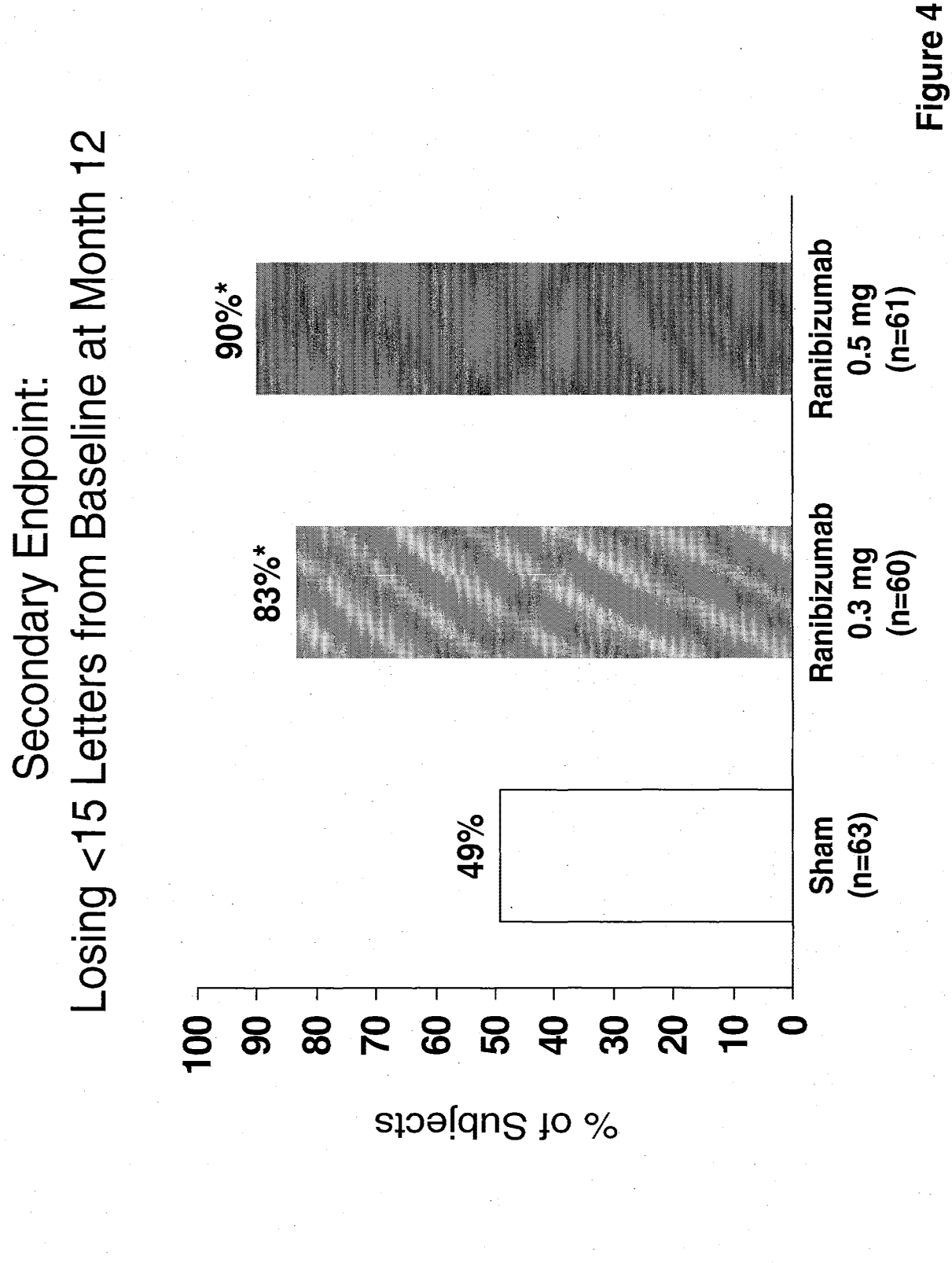

Secondary Endpoint:
Losing <15 Letters from Baseline at Month 12
% of Subjects
100
90
80
70
60
50
40
30
20
10
0
49%
83%*
90%*
Sham (n=63)
Ranibizumab 0.3 mg (n=60)
Ranibizumab 0.5 mg (n=61)
* P <0.0001 vs sham

Figure 4

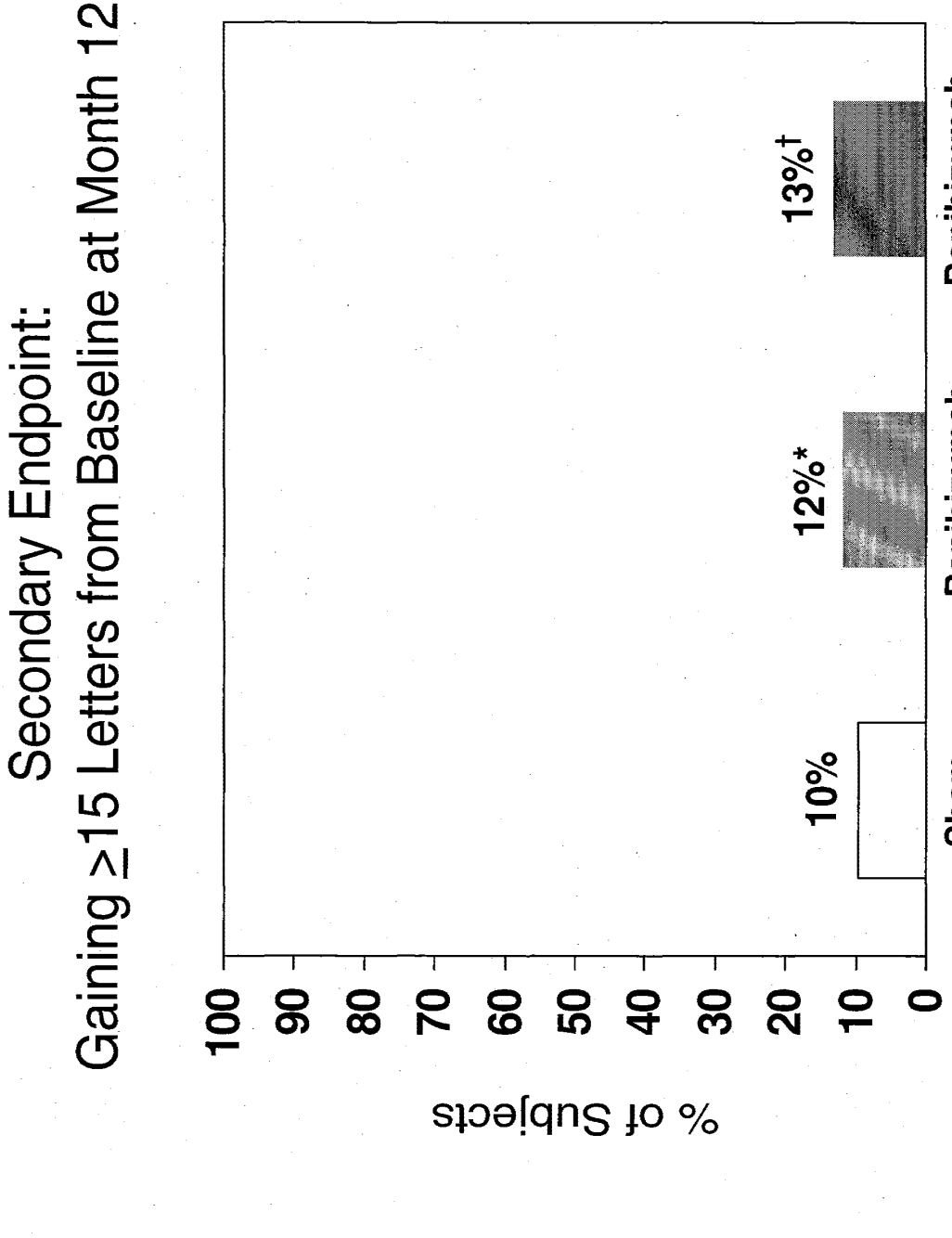


Figure 5

*P=0.87 vs sham; †P=0.71 vs sham

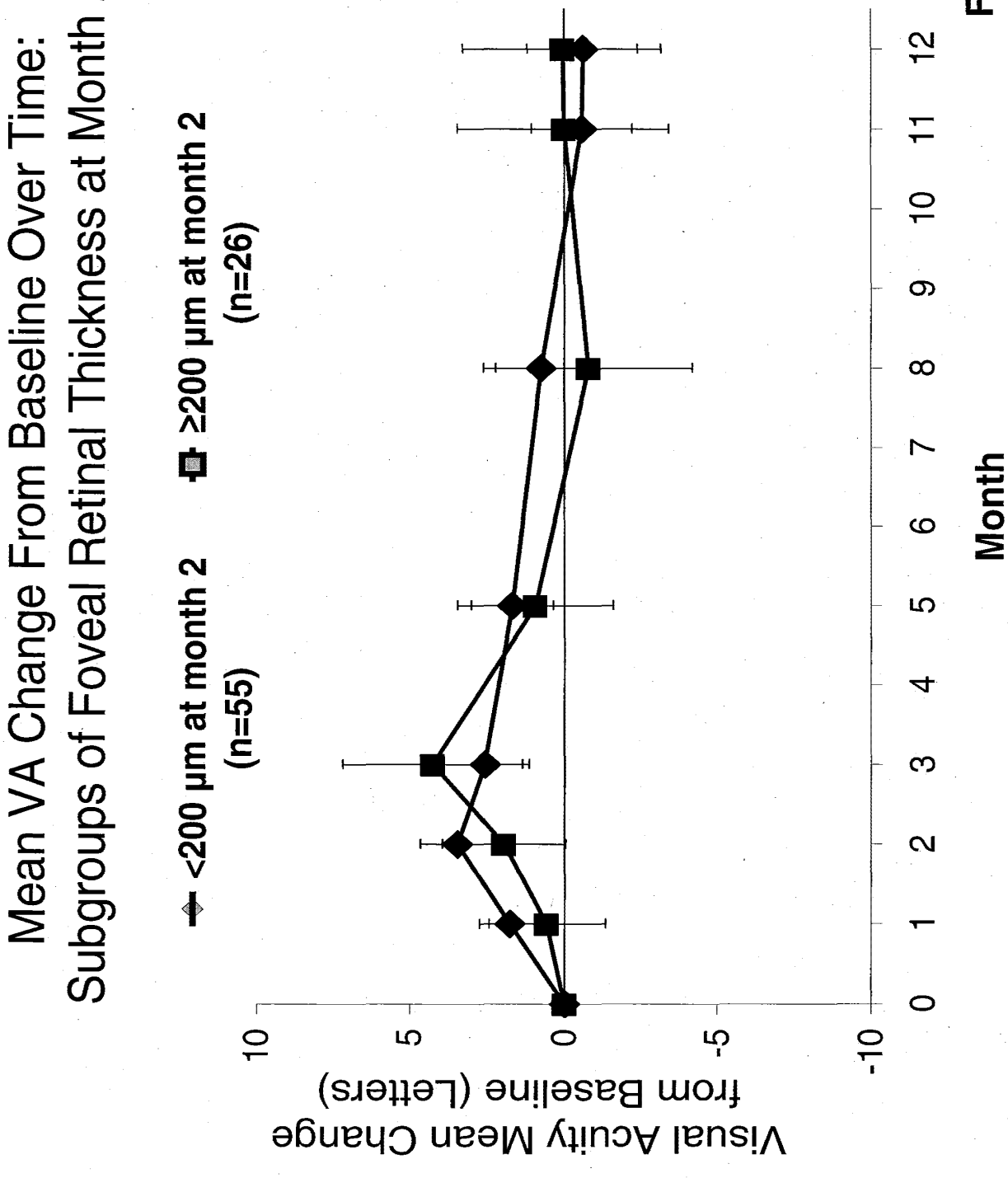


Figure 6

Note: Vertical bars are ± one standard error of the mean.

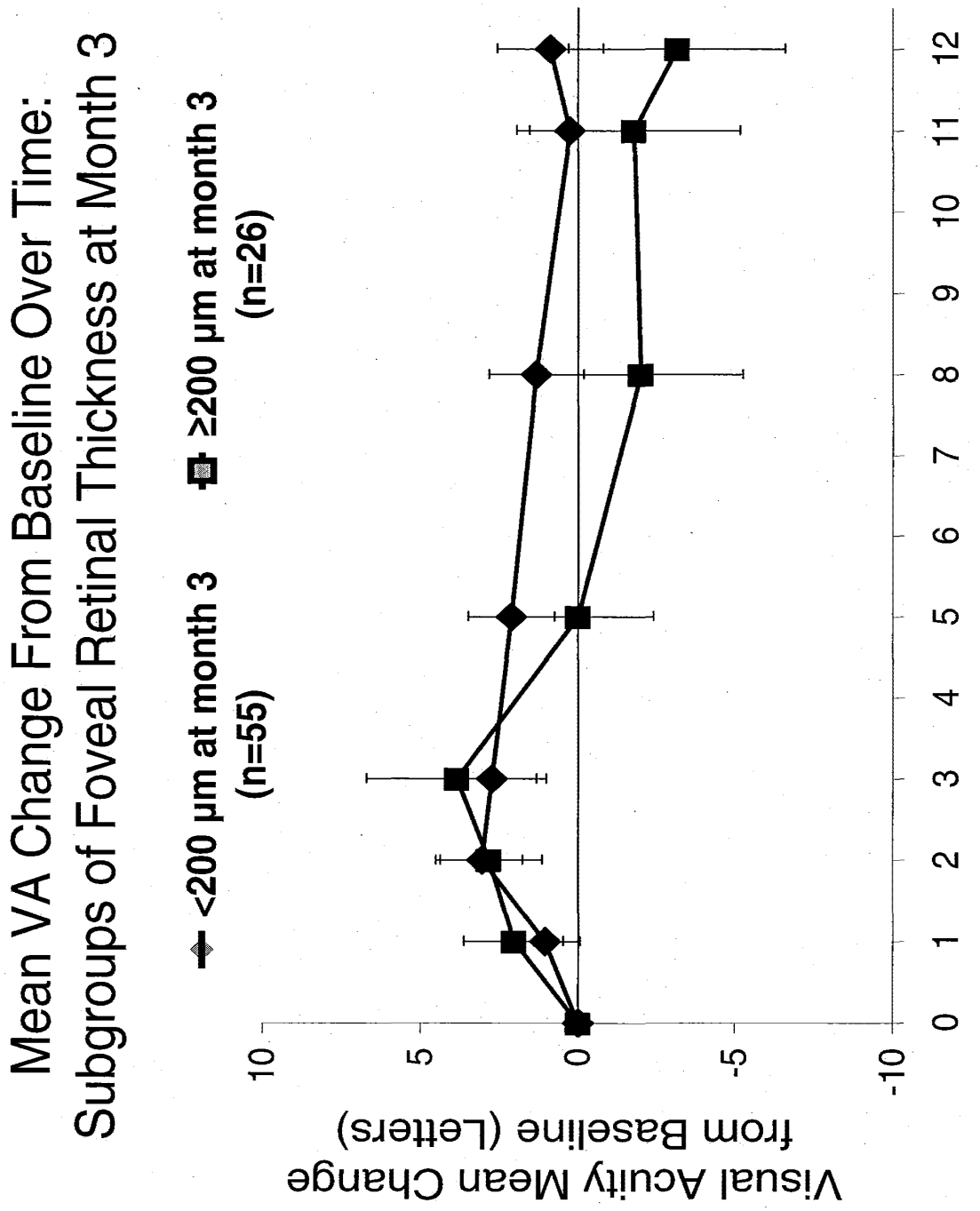


Figure 7

Note: Vertical bars are ± one standard error of the mean.

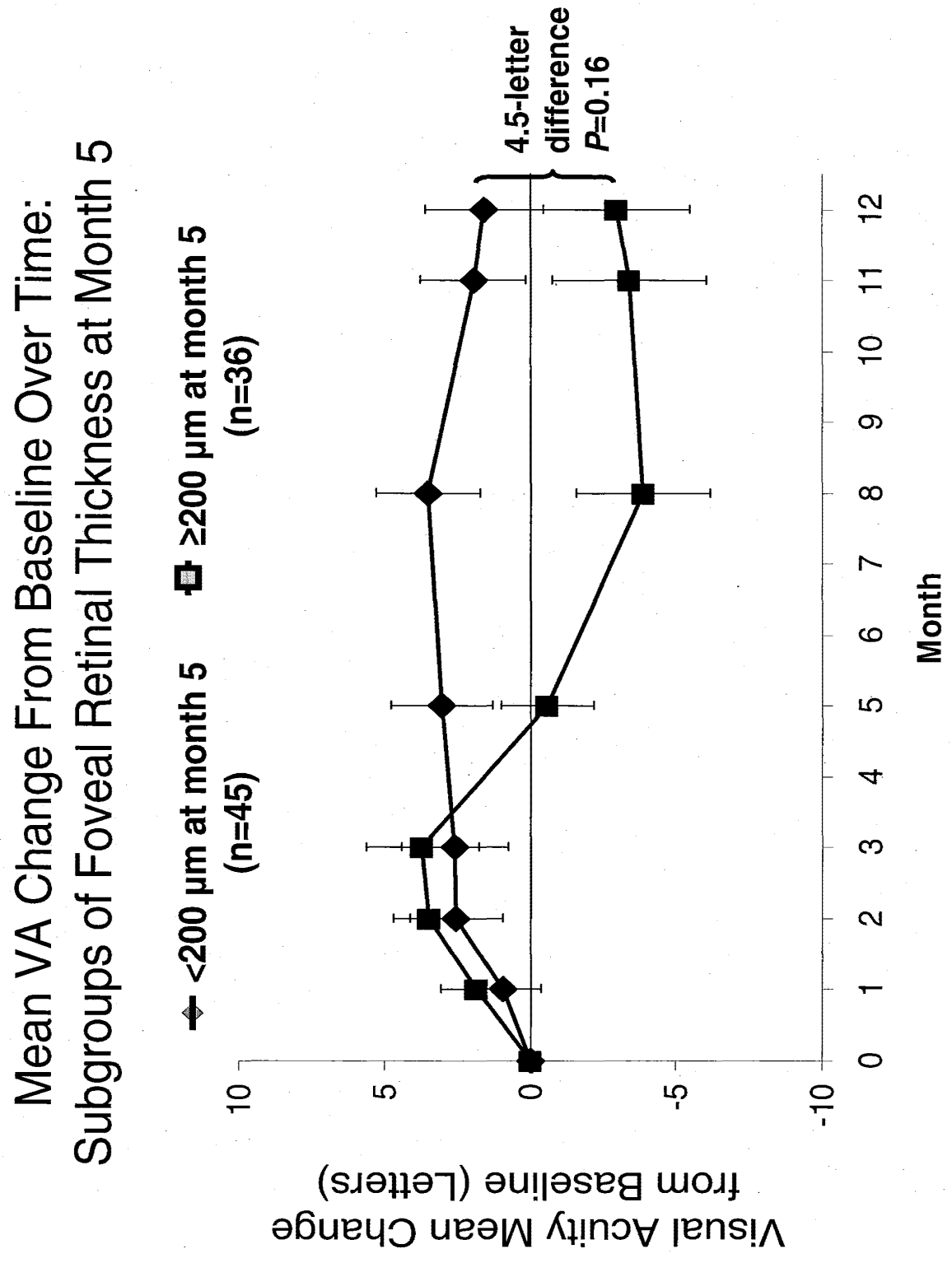


Figure 8

Note: Vertical bars are ± one standard error of the mean.

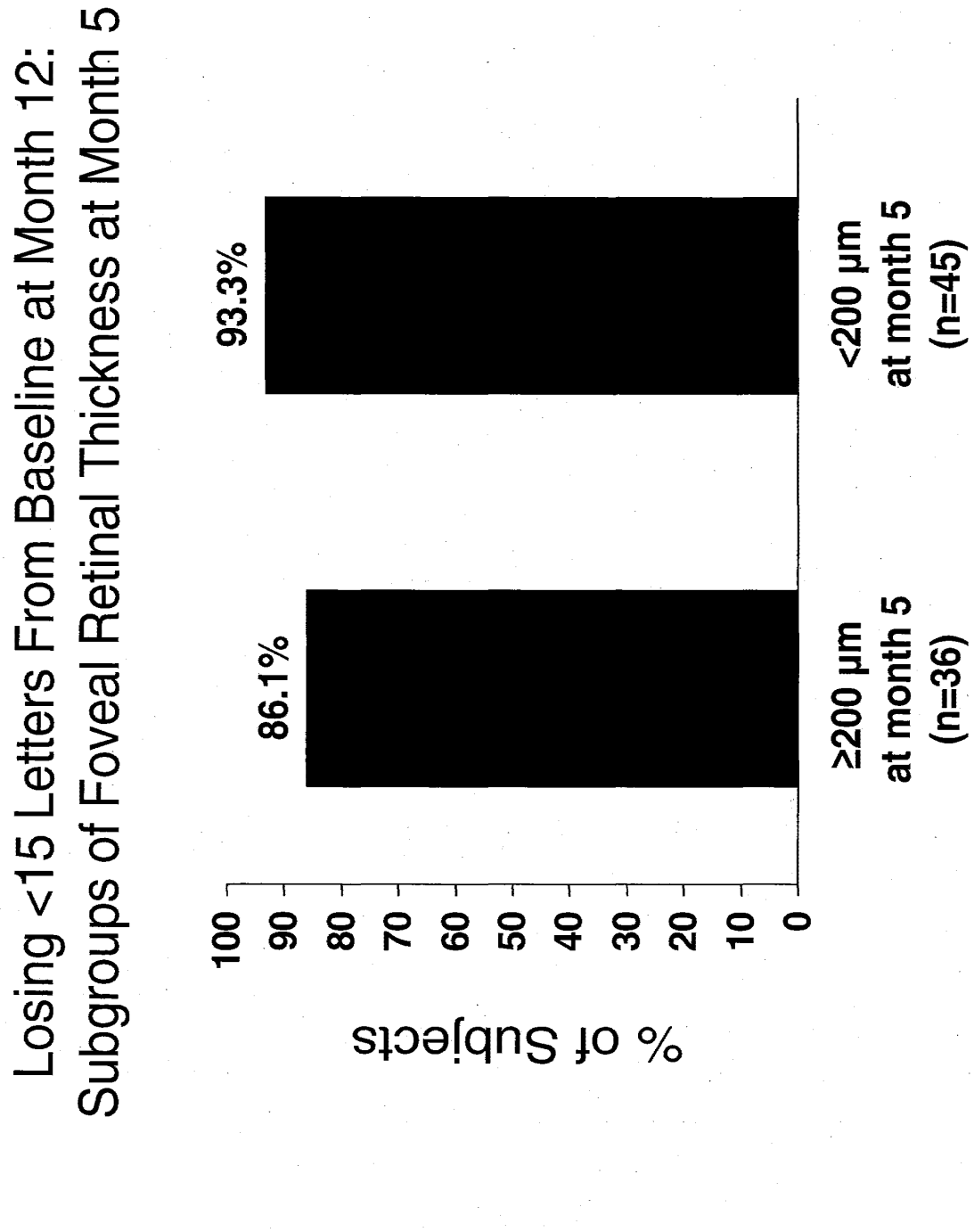

Losing <15 Letters From Baseline at Month 12:
Subgroups of Foveal Retinal Thickness at Month 5
% of Subjects
100
90
80
70
60
50
40
30
20
10
0
86.1%
93.3%
≥200 µm at month 5 (n=36)
<200 µm at month 5 (n=45)
P =0.28 for comparing two groups

Figure 9

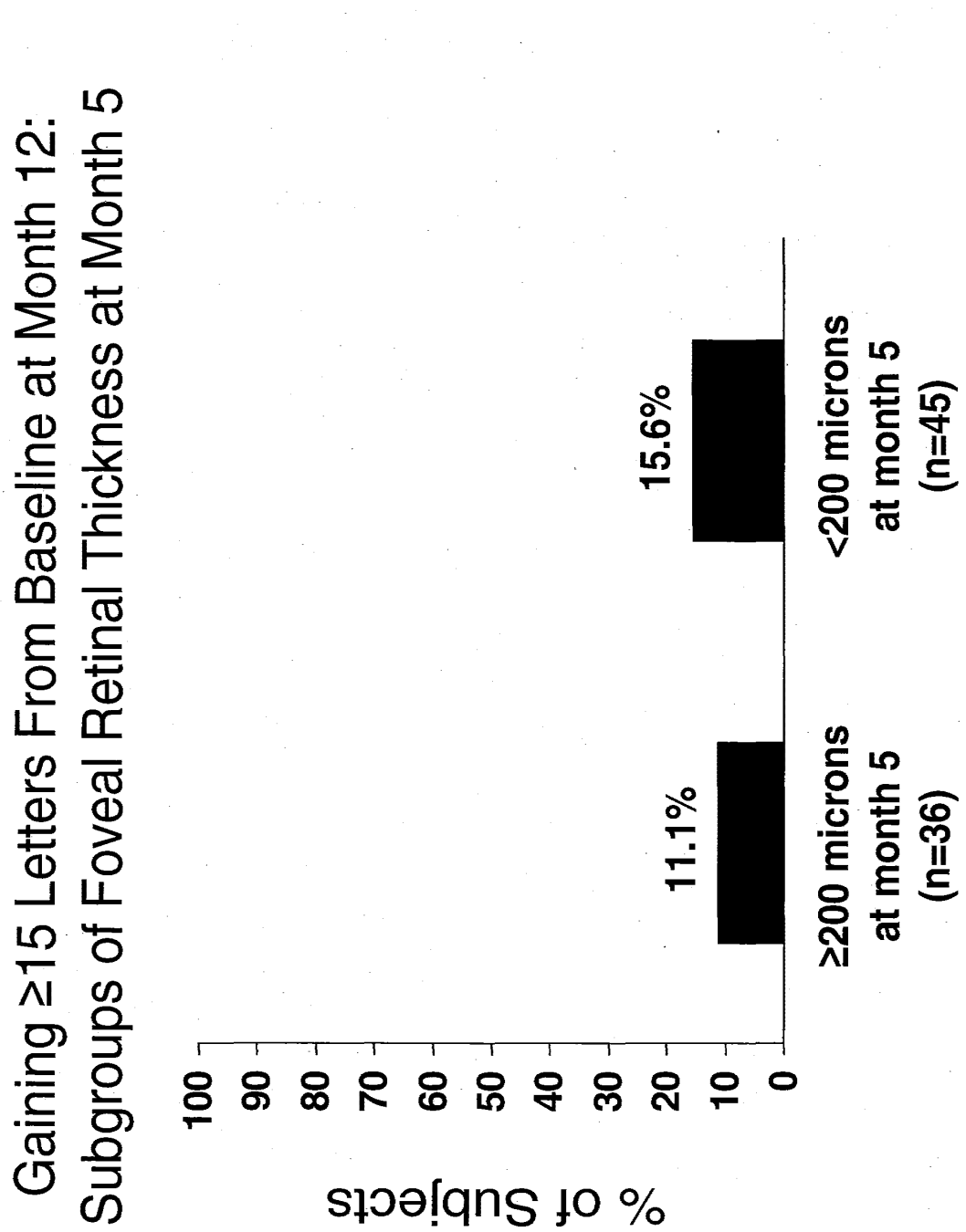


Figure 10

P =0.56 for comparing two groups

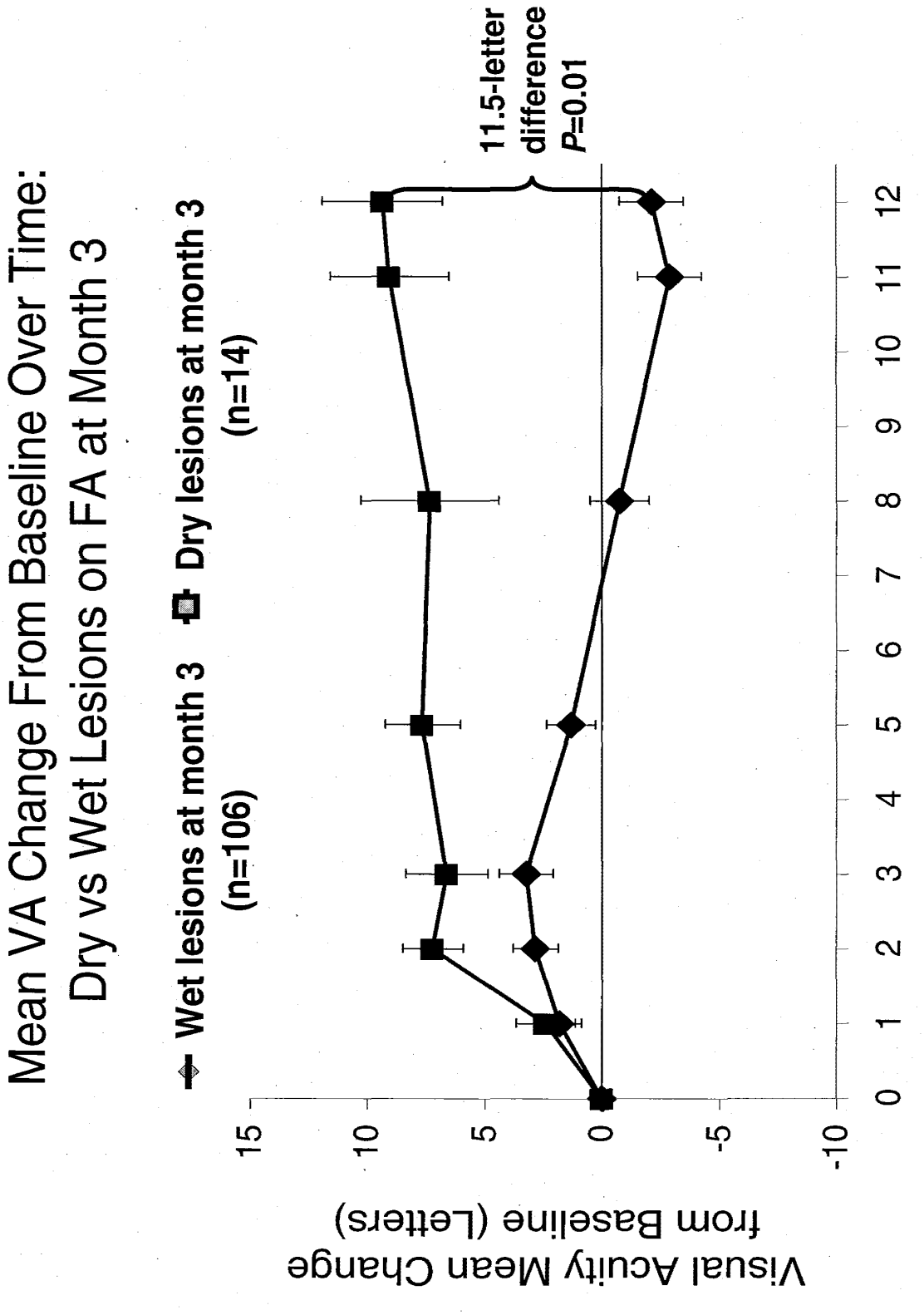


Figure 11

Note: Vertical bars are ± one standard error of the mean.

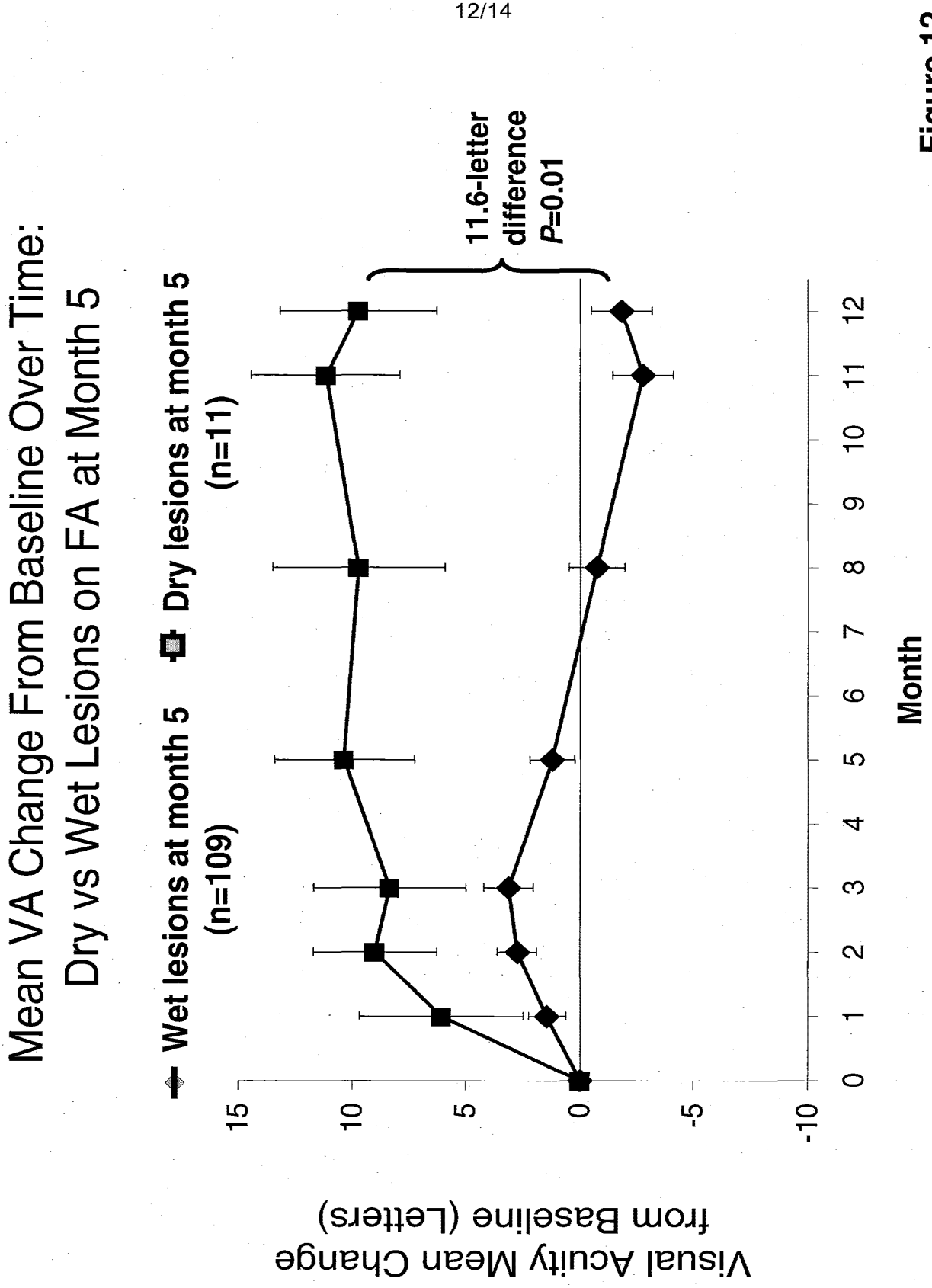
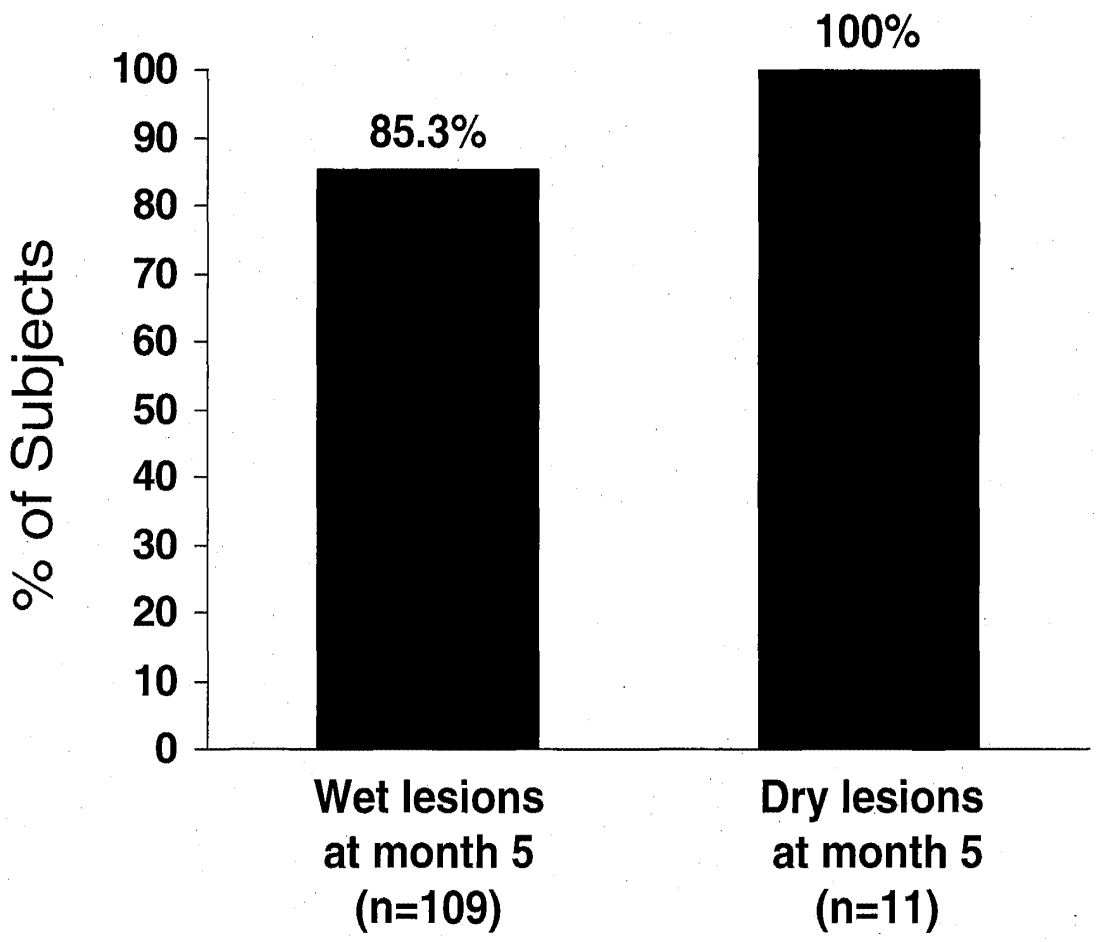


Figure 12

Note: Vertical bars are ± one standard error of the mean.



P=0.17 for comparing two groups

Figure 13

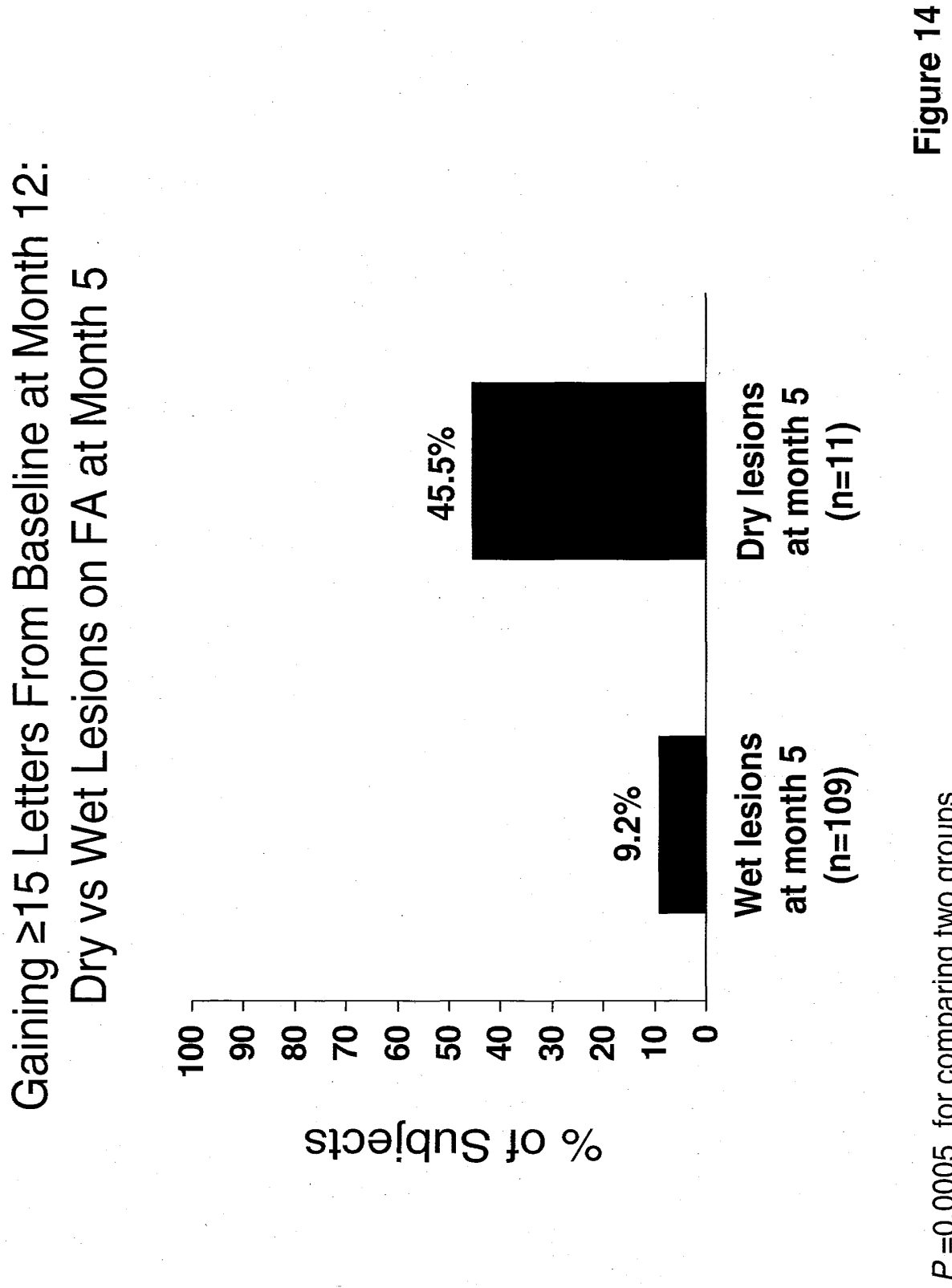


Figure 14

P =0.0005 for comparing two groups

Electronic Patent Application Fee Transmittal	
Application Number:	13940370
Filing Date:	12-Jul-2013
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Filer:	Karl Bozicevic/Kimberly Zuehlke
Attorney Docket Number:	REGN-008CIP (725A1-US)
Filed as Large Entity	
Filing Fees for Utility under 35 USC 111(a)	

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:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt	
EFS ID:	24134036
Application Number:	13940370
International Application Number:	
Confirmation Number:	1055
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-008CIP (725A1-US)
Receipt Date:	19-NOV-2015
Filing Date:	12-JUL-2013
Time Stamp:	15:41:51
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$180
RAM confirmation Number	2274
Deposit Account	
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The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	REGN-008CIP_11-19-2015_supp_IDS_trans.pdf	51451 2ef6dd34bd428065d70294dacf00e22ed4b085f4	no	3
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	REGN-008CIP_11-19-2015_Supp_IDS.pdf	612549 c758898f79db9966594bfed610793e05586c4206	no	4
Warnings:					
Information:					
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3	Foreign Reference	JP2010509369.pdf	329513 1ec9d57004a34e46f900995ce23afffd57e8e83a	no	22
Warnings:					
Information:					
4	Foreign Reference	WO08063932.pdf	2382698 c5553f9daa9af3667184854923c43b41897e41e4	no	47
Warnings:					
Information:					
5	Non Patent Literature	NGUYEN_2006.pdf	1241838 3a523072f97e356261a807eb0450ea60e0239ace	no	15
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Information:					
		Total Files Size (in bytes):	4649099		

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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.

Electronically Filed 11/19/2015

INFORMATION DISCLOSURE STATEMENT Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450		
	Attorney Docket	REGN-008CIP
	First Named	YANCOPOULOS, GEORGE D.
	Application Number	13/940,370
	Confirmation No.	1055
	Filing Date	July 12, 2013
	Group Art Unit	1647
	Examiner Name	LOCKARD, JON MCCLELLAND
	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

Applicants submit herewith documents which may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 C.F.R. § 1.56. This submission is not intended to constitute an admission that any document referred to therein is "prior art" for this invention unless specifically designated as such. A listing of the documents is shown on enclosed Form PTO/SB/08A and copies of the foreign patents and non-patent literature are also enclosed.

The Examiner is requested to make the documents listed on the enclosed PTO/SB/08A of record in this application. Applicants would appreciate the Examiner initialing and returning the initialed copy of form PTO/SB/08A, indicating the document cited therein have been considered and made of record herein.

Statements

☐ **No statement**

☒ **PTA Statement under 37 CFR § 1.704(d)(1):** Each item of information contained in the information disclosure statement filed herewith:

(i) Was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office, and this communication was not received by any individual designated in § 1.56(c) more than thirty days prior to the filing of the information disclosure statement; or

(ii) Is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office, and this communication

was not received by any individual designated in § 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

☒ **IDS Statement under 37 CFR § 1.97(e)(1):** 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; or

☐ **IDS Statement under 37 CFR § 1.97(e)(2):** 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 § 1.56(c) more than three months prior to the filing of the information disclosure statement.

Fees

☐ No fee is believed to be due.

☒ The appropriate fee set forth in 37 C.F.R. §1.17(p) accompanies this information disclosure statement.

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-008CIP.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: November 19, 2015

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
Reg. No. 28,807

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/940,370	07/12/2013	George D. YANCOPOULOS	REGN-008CIP (725A1-US)	1055

96387 7590 12/10/2015

Regeneron - Bozicevic, Field & Francis
1900 University Ave
Suite 200
East Palo Alto, CA 94303

EXAMINER
LOCKARD, JON MCCLELLAND

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
12/10/2015	ELECTRONIC

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.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docket@bozpat.com

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APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR / PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
13/940,370	12 July, 2013	YANCOPOULOS, GEORGE D.	REGN-008CIP (725A1-US)

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East Palo Alto, CA 94303

EXAMINER	
JON M. LOCKARD	
ART UNIT	PAPER
1647	20151204

DATE MAILED:

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Commissioner for Patents

1. The information disclosure statement (IDS) submitted on 19 November 2015 was filed after the mailing date of the Notice of Allowance on 19 October 2015. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

/JON M LOCKARD/
Examiner, Art Unit 1647

PTO-90C (Rev.04-03)

Receipt date: 11/19/2015
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	13940370
Filing Date	2013-07-12
First Named Inventor	George D. YANCOPOULOS
Art Unit	1647
Examiner Name	LOCKARD, Jon McClelland
Attorney Docket Number	REGN-008CIP

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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
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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	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]
/J.L./	1	2010-509369	JP		2010-03-25	Genentech, Inc.	See WO 2008/063932 for English Equivalent	☒
/J.L./	2	2008/063932	WO		2008-05-29	Genentech, Inc.		☐

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Receipt date: 11/19/2015		
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Application Number	13940370
	Filing Date	2013-07-12
	First Named Inventor	George D. YANCOPOULOS
	Art Unit	1647
	Examiner Name	LOCKARD, Jon McClelland
	Attorney Docket Number	REGN-008CIP

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]
/J.L./	1	NGUYEN et al., "A phase I trial of an IV-administered vascular endothelial growth factor trap for treatment in patients with choroidal neovascularization due to age-related macular degeneration" Ophthalmology (Sept 2006) 113 (9):1522e1-1522e14 (epub July 28,2006)	☐

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EXAMINER SIGNATURE

Examiner Signature	/Jon Lockard/	Date Considered	12/04/2015

*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.

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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)

96387 7590 10/19/2015

Regeneron
Bozicevic, Field & Francis
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East Palo Alto, CA 94303

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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.

Kimberly W Zuehlke	(Depositor's name)
/Kimberly W Zuehlke/	(Signature)
18 December 2015	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/940,370	07/12/2013	George D. YANCOPOULOS	REGN-008CIP (725A1-US)	1055

TITLE OF INVENTION: USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	01/19/2016

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 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Frank Cottingham
2 Karl Bozicevic
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, New York

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

☐ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

☐ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

☐ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Karl Bozicevic, Reg. No. 28,807/ Date 18 December 2015

Typed or printed name Karl Bozicevic Registration No. 28,807

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Electronic Patent Application Fee Transmittal

Application Number:	13940370
Filing Date:	12-Jul-2013
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Filer:	Karl Bozicevic/Kimberly Zuehlke
Attorney Docket Number:	REGN-008CIP (725A1-US)

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

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	960	960

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**960**

Electronic Acknowledgement Receipt	
EFS ID:	24408050
Application Number:	13940370
International Application Number:	
Confirmation Number:	1055
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-008CIP (725A1-US)
Receipt Date:	18-DEC-2015
Filing Date:	12-JUL-2013
Time Stamp:	12:59:12
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
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Payment was successfully received in RAM	$960
RAM confirmation Number	11160
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	REGN-008CIP_Issue_fee.pdf	1121775 8b9f3a5ca823816fd5cc89edfd249ba76cf0e25a	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	31176 bb7d7a48a44c2964d89dc53537fb7cbc162252da	no	2
Warnings:					
Information:					
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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.

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<u>New International Application Filed with the USPTO as a Receiving Office</u>
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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/940,370	02/09/2016	9254338	REGN-008CIP (725A1-US)	1055

96387 7590 01/20/2016

Regeneron - Bozicevic, Field & Francis
1900 University Ave
Suite 200
East Palo Alto, CA 94303

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 132 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):

REGENERON PHARMACEUTICASS, INC., Tarrytown, NY;
George D. YANCOPOULOS, Yorktown Heights, NY;

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