DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 17072417 Document Date: 10/16/2020

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

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

- Sequence Listing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via DAV or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: March 1, 2019

Electronically Filed

PRELIMINARY AMENDMENT Under CFR 1.115 Address to: Mail Stop Patent Application Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Attorney Docket No.	REGN-008CIPCON6
	Confirmation No.	To Be Assigned
	First Named Inventor	YANCOPOULOS, GEORGE D.
	Application Number	To Be Assigned
	Filing Date	Herewith
	Group Art Unit	
	Examiner Name	
	Title: *"Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders"*	

Sir:

Prior to the examination of the above-referenced application on the merits, please enter the amendments below.

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:	Karl Bozicevic/Kimberly Zuehlke
Attorney Docket Number:	REGN-008CIPCON6

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
UTILITY APPLICATION FILING	1011	1	320	320
UTILITY SEARCH FEE	1111	1	700	700
UTILITY EXAMINATION FEE	1311	1	800	800
Pages:				
Claims:				
CLAIMS IN EXCESS OF 20	1202	32	100	3200
Miscellaneous-Filing:				
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**5020**

Electronic Acknowledgement Receipt

EFS ID:	40864828
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	16-OCT-2020
Filing Date:	
Time Stamp:	13:59:34
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$5020
RAM confirmation Number	E20200FD59579152
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	Application Data Sheet	WebADS.pdf	143341 2923394182dacb29dea36a7a5aca4618c6de1442	no	8
Warnings:					
Information:					
2		REGN-008CIPCON6_2020-10-16_Appln_as_fld.pdf	159372 5a60e89dc83c584ba38225342c137aa4a20283d2	yes	25

Multipart Description/PDF files in .zip description

Document Description	Start	End
Abstract	25	25
Claims	23	24
Specification	1	22

Warnings:					
Information:					
3	Drawings-only black and white line drawings	REGN-008CIPCON6_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:					
4	Oath or Declaration filed	REGN-008CIPCON6_declaration.pdf	173097 6bda7272374e6af80c8c3d8cf30d012e4657b588	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:					

5	Transmittal Letter	REGN-008CIPCON6_2020-10-16_IDS_Trans.pdf	52926 5d144817290af42b12711d58c36c67062ae79109	no	3
Warnings:					
Information:					
6	Information Disclosure Statement (IDS) Form (SB08)	REGN-008CIPCON6_2020-10-16_IDS_SB08A.pdf	194906 936a86e4f82aa53a16ef5ef908b6f7fc573bea1b	no	18
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
7		REGN-008CIPCON6_2020-10-16_pre_amend.pdf	77309 713a82d721e278305dd15219b3eb58af5fb056c5	yes	10

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Applicant Arguments/Remarks Made in an Amendment	8	10
	Claims	2	7
	Preliminary Amendment	1	1

Warnings:					
Information:					
8	Sequence Listing (Text File)	REGN-008CIPCON6_SeqList.txt	6359	no	-
Warnings:					
Information:					
9	Fee Worksheet (SB06)	fee-info.pdf	38541 8419c46bd61329de183aa14d84168d2205768afc	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	951244		

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.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON6
	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

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	George	D.	YANCOPOULOS	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Yorktown Heights	State/Province	NY	Country of Residence i	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	**State/Province**	NY
Postal Code	10591	**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	docket@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	REGN-008CIPCON6	**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 (08-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON6
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

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	93726		

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix	Remove
Registration Number					
Prefix	**Given Name**	Middle Name	**Family Name**	Suffix	Remove
Registration Number					

Additional Representative Information blocks may be generated within this form by selecting the **Add** button.

PTO/AIA/14 (08-15)
Approved for use through 04/30/2017. 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	REGN-008CIPCON6
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim 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.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	16055847	2018-08-06

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	16397267	2019-04-29

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
16397267	Continuation of	16159282	2018-10-12

Prior Application Status	Patented				Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
16159282	Continuation of	15471506	2017-03-28	10130681	2018-11-20

Prior Application Status	Patented				Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15471506	Continuation of	14972560	2015-12-17	9669069	2017-06-06

Prior Application Status	Patented				Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
14972560	Continuation of	13940370	2013-07-12	9254338	2016-02-09

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
13940370	Continuation in part of	PCT/US2012/020855	2012-01-11

PTO/AIA/14 (08-15)
Approved for use through 04/30/2017. 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	REGN-008CIPCON6
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2012/020855	Claims benefit of provisional	61432245	2011-01-13

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2012/020855	Claims benefit of provisional	61434836	2011-01-21

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2012/020855	Claims benefit of provisional	61561957	2011-11-21

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

Foreign Priority Information:

This section allows for the applicant to claim 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. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(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).

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.

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.

PTO/AIA/14 (08-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON6
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is 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 State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

PTO/AIA/14 (08-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON6
	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

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 For Applicant:

Address 1	777 Old Saw Mill River Road		
Address 2			
City	Tarrytown	**State/Province**	NY
Country[i]	US	Postal Code	10591
Phone Number		Fax Number	
Email Address			

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

PTO/AIA/14 (08-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON6
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

Assignee Information including Non-Applicant Assignee 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.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☒

Organization Name	REGENERON PHARMACEUTICALS, INC.

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1	777 Old Saw Mill River Road		
Address 2			
City	Tarrytown	**State/Province**	NY
Country i	US	Postal Code	10591
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON6
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e. g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Karl Bozicevic/			Date (YYYY-MM-DD)	
First Name	Karl	Last Name	Bozicevic	Registration Number	28807

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

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.

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.

USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of 16/055,847 filed August 6, 2018 and is a continuation of 16/397,267 filed April 29, 2019 which is a continuation 16/159,282 filed October 12, 2018, which is a continuation of 15/471,506 filed March 28, 2017, now U.S. Patent No. 10,130,681 issued November 20, 2018, which is a continuation of 14/972,560 filed December 17, 2015, now U.S. Patent No. 9,669,069 issued June 6, 2017, which is a continuation of 13/940,370 filed July 12, 2013, now U.S. Patent No. 9,254,338 issued February 9, 2016, which 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 weeks[a] (2Q8)	42	8.5**	9.7**
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):

ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGTCTGCTTCTCAC
AGGATCTAGTTCCGGAAGTGATACCGGTAGACCTTTCGTAGAGATGTACAGTGAAATCCCCGA
AATTATACACATGACTGAAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCACCTAACAT
CACTGTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGCATAATCTGG
GACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGCTTCTGACCTGT
GAAGCAACAGTCAATGGGCATTTGTATAAGACAAACTATCTCACACATCGACAAACCAATACAA
TCATAGATGTGGTTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTGGAGAAAAGCTTGTCTT
AAATTGTACAGCAAGAACTGAACTAAATGTGGGGATTGACTTCAACTGGGAATACCCTTCTTCG
AAGCATCAGCATAAGAAACTTGTAAACCGAGACCTAAAAACCCAGTCTGGGAGTGAGATGAAG
AAATTTTTGAGCACCTTAACTATAGATGGTGTAACCCGGAGTGACCAAGGATTGTACACCTGTG
CAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACATTTGTCAGGGTCCATGAAAAGGACA
AAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGGACCGTCAGTCTTCCTCT
TCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTG
GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGT
GCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG
TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC
AAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACC
ACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCT
GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCG
GAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC
AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

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

MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLK
KFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGI
ELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRS
DQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV
TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[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.

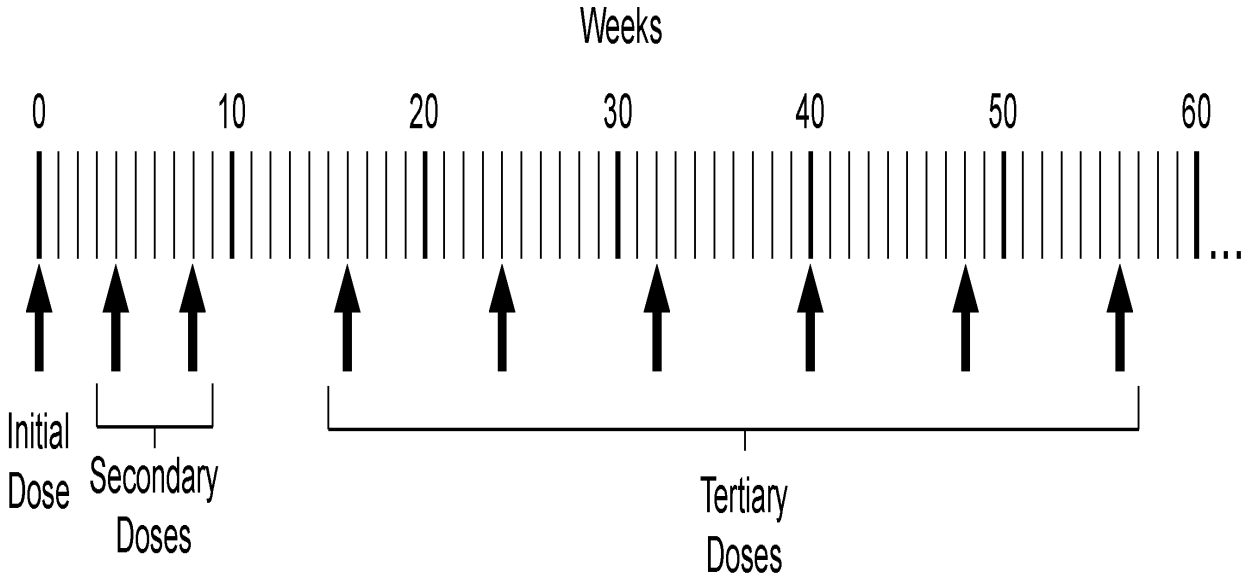

Weeks
0
10
20
30
40
50
60
...
Initial Dose
Secondary Doses
Tertiary Doses

Figure 1

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.

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.

Electronically Filed 10/16/2020

INFORMATION DISCLOSURE STATEMENT	Attorney Docket No.	REGN-008CIPCON6
	Confirmation No.	To Be Assigned
	First Named Inventor	George D. Yancopoulos
	Application Number	To Be Assigned
	Filing Date	October 16, 2020
	Group Art Unit	To Be Assigned
	Examiner Name	To Be Assigned
Address to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

Applicant submits 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.

The publications discussed herein are provided to comply with the duty to disclose in accordance with 37 C.F.R. § 1.56. However, nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed

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 documents cited therein have been considered and made of record herein.

All of the references identified herein were disclosed in parent application serial number 16/397,267, and as such, copies thereof are not included pursuant to the provisions of 37 CFR § 1.98(d).

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-008CIPCON6.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: October 16, 2020

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
Reg. No. 28,807

BOZICEVIC, FIELD & FRANCIS LLP
201 Redwood Shores Parkway, Suite 200
Redwood City, CA 94065
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	1	of	18	Attorney Docket Number	REGN-008CIPCON6

U.S. PATENT DOCUMENTS

Examiner Initial*	Cite No.	Patent Number Number-Kind Code (*if known*)	Issue Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	7070959	2006-07-04	Papadopoulos	
	2	7303746	2007-12-04	Wiegand	
	3	7303748	2007-12-04	Wiegand	
	4	7306799	2007-12-11	Wiegand	
	5	7396664	2008-07-08	Daly et al.	
	6	8092803	2012-01-10	Furfine et al.	
	7	9254338	2016-02-09	Yancopoulos	
	8	9669069	2017-06-06	Yancopoulos	
	9	10130681	2018-11-20	Yancopoulos	
	10	10406226	2019-09-10	Dix et al.	
	11	10464992	2019-11-05	Furfine et al.	

U.S. PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No.	Publication Number **Number-Kind Code** (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	2003/0171320	2003-09-11	Guyer	
	2	2005/0163798	2005-07-28	Papadopoulos et al.	
	3	2005/0260203	2005-11-24	Wiegand et al.	
	4	2006/0058234	2006-03-16	Daly et al.	
	5	2006/0172944	2006-08-03	Wiegand et al.	
	6	2007/0190058	2007-08-16	Shams	
	7	2008/0220004	2008-09-11	Wiegand et al.	
	8	2019/0290725	2019-09-26	Vitti et al.	
	9	2019/0388539	2019-12-26	Dix et al.	
	10	2020/0017572	2020-01-16	Furfine et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No.	Foreign Document Number Country Code-Number-Kind Code (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	1	WO 2006/047325	2006-03-04	Genentech, Inc.		
	2	WO 2000/75319	2000-12-14	Regeneron Pharmaceuitcals, Inc.		
	3	WO 2004/106378 A2	2004-12-09	Regeneron Pharmaceuticals, Inc.		
	4	WO 2005/000895 A2	2005-01-05	Regeneron Pharmaceuticals, Inc.		
	5	WO 2007/022101 A2	2007-02-22	Regeneron Pharmaceuticals, Inc.		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	2	of	18	Attorney Docket Number	REGN-008CIPCON6

FOREIGN PATENT DOCUMENTS						
Examiner Initial*	Cite No.	Foreign Document Number Country Code-Number-Kind Code (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	6	WO 2008/063932	2008-05-29	Genentech, Inc.		
	7	JP 2010-509369	2010-03-25	Genentech, Inc.	See WO 2008/063932 for English Equivalent	
	8	WO 2012/097019	2012-07-19	Regeneron Pharmaceuticals, Inc.		

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	1	16/055,847 – Third Party Submissions dated May 1, 2019		
	2	16/159,282 – Third Party Submissions dated May 31, 2019		
	3	ADSIS R&D Profile "Aflibercept: AVE 0005, AVE 005, AVE0005, VEGF Trap - Regeneron, VEGF Trap (R1R2), VEGF Trap-Eye." Drugs R D, 9(4):261-269 (2008)		
	4	ANONYMOUS "Lucentis (rangibizymab injection) Intravitreal Injection" pp. 103 (June 2006)		
	5	ANONYMOUS "Anti-VEGF 2019: The State of the Art" Review of Ophthalmology (published August 5, 2019)		
	6	BARBAZETTO, "Dosing Regimen And The Frequency Of Macular Hemorrhages In Neovascular Age-Related Macular Degeneration Treated With Ranibizumab." Retina, 30(9):1376-85 (2010)		
	7	Bayer Investor News, "Bayer and Regeneron Start additional Phase 3 Study for VEGF Trap-Eye in Wet Age-related Macular Degeneration." (May 8, 2008)		
	8	Bayer Investor News, "VEGF Trap-Eye: New Data Confirm Successes in the Treatment of Age-related Macular Degeneration" (September 28, 2008)		
	9	BENZ et al. "CLEAR-IT-2: Interim Results Of The Phase II, Randomized, Controlled Dose- and Interval-ranging Study Of Repeated Intravitreal VEGF Trap Administration In Patients With Neovascular Age-related Macular Degeneration (AMD)" ARVO Annual Meeting Abstract (May 2007)		
	10	BOYER, "A Phase IIIb Study to Evaluate the Safety of Ranibizumab in Subjects with Neovascular Age-related Macular Degeneration." Ophthalmology, 116(9):1731-39 (September 2009)		
	11	BROWN, "Ranibizumab versus Verteporfin for Neovascular Age-Related Macular Degeneration." N Engl J Med, 355(14):1432-44 (October 5, 2006)		
	12	BROWN, "Primary Endpoint Results of a Phase II Study of Vascular Endothelial Growth Factor Trap-Eye in Wet Age-related Macular Degeneration." Ophthalmology, 118(6):1089-97 (June 2011)		
	13	BROWN, "Long-term Outcomes of Ranibizumab Therapy for Diabetic Macular Edema: The 36-Month Results from Two phase III Trials." Ophthalmology, 120(10):2013-22 (October 2013)		
	14	BROWNING et al. "Aflibercept for age-related macular degeneration: a game-changer or quiet addition?" American Journal of Ophthalmology, 154(2):222-226 (August 2012)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	3	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	15	CAMPOCHIARO et al. "Ranibizumab for Macular Edema Due to Retinal Vein Occlusions Implication of VEGF as a Critical Stimulator" Molecular Therapy, 16(4):791-799 (2008)		
	16	CAMPOCHIARO, "Ranibizumab for Macular Edema following Branch Retinal Vein Occlusion: six-month primary end point results of a phase III study." Ophthalmology, 117(6):1102-1112 (June 2010)		
	17	CAMPOCHIARO, "Sustained Benefits from Ranibizumab for Macular Edema following Central Retinal Vein Occlusion: Twelve-Month Outcomes of a phase III Study." Ophthalmology, 188(10):2041-49 (October 2011)		
	18	CAO, "A Subretinal Matrigel Rat Choroidal Neovascularization (CNV) Model and Inhibition of CNV and Associated Inflammation and Fibrosis by VEGF Trap" Investigative Ophthalmology & Visual Science, 51(11):6009- 6017 (November 2010)		
	19	CENTER FOR DRUG EVALUATION AND RESEARCH APPLICATION NUMBER: 21-756 MEDICAL REVIEW(S) (December 17, 2004) <URL:https://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-756_Macugen_medr.pdf>		
	20	CENTER FOR DRUG EVALUATION AND RESEARCH BLA APPLICATION NUMBER: 125156 MEDICAL REVIEW, (June 2006) <URL:https://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/125156s000_Lucentis_MedR.pdf>		
	21	CHARLES, Steve (Guest Lecturer) "VEGF Trap Has Positive DME Data" Tenth Annual Retina Fellows Forum Jan 29 and 30, Chicago, (Article Date 03/01/2010)		
	22	CHATZIRALLI et al. "Intravitreal aflibercept for neovascular age-related macular degeneration in patients aged 90 years or older: 2-year visual acuity outcomes" Eye (2018) 32:1523-1529		
	23	CHUNG et al. "Ziv-aflibercept: A novel angiogenesis inhibitor for the treatment of metastatic colorectal cancer" Am J Heath-Syst Pharm (November 1, 2013) 70:1887-1896		
	24	COOPER et al., "Increased Renal Expression of Vascular Endothelial Growth Factor (VEGF) and Its Receptor VEGFR-2 in Experimental Diabetes" Diabetes (1999) 48:2229-2239		
	25	CROLL et al., "VEGF-mediated inflammation precedes angiogenesis in adult brain" Experimental Neurology (2004) 187:388-402		
	26	CSAKY, "Safety Implications of Vascular Endothelial Growth Factor Blockade for Subjects Receiving Intravitreal Anti-Vascular Endothelial Growth Factor Therapies." Am. J. Ophthalmology, 148(5):647-56, (November 2009)		
	27	DeVRIESE et al., "Antibodies against Vascular Endothelial Growth Factor Improve Early Renal Dysfunction in Experimental Diabetes" J. Am. Soc. Nephrol (2001) 12:993-1000		
	28	DIXON et al., "VEGF Trap-Eye for the treatment of neovascular age-related macular degeneration" Expert Opin. Investig. Drugs, 18(10):1573-1580 (2009)		
	29	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 Ophthalmol. 93(2):144-1449 (February 2009)		
	30	DO et al., "The DA VINCI Study: phase 2 primary results of VEGF Trap-Eye in patients with diabetic macular edema" Ophthalmology, 118(9):1819-1826 (September 2011)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	4	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	31	DO, "One-Year Outcomes of the DA VINCI Study of VEGF Trap-Eye in Eyes with Diabetic Macular Edema." Ophthalmology, 119(8):1658-65 (2012)		
	32	DO et al. "Results of a Phase 1 Study of Intravitreal VEGF Trap in Subjects with Diabetic Macular Edema: The CLEAR-IT DME Study" ARVO Annual Meeting Abstract (May 2007)		
	33	DO et al. "VEGF Trap-Eye Vision-specific Quality of Life through 52 Weeks in Patients with Neovascular AMD in CLEAR-IT 2: A Phase 2 Clinical Trial" ARVO Annual Meeting Abstract (April 2009)		
	34	EICHTEN, "Rapid decrease in tumor perfusion following VEGF blockade predicts long-term tumor growth inhibition in preclinical tumor models" Angiogenesis, 16:429-441 (2013)		
	35	ENGELBERT, "Treat And Extend' Dosing Of Intravitreal Antivascular Endothelial Growth Factor Therapy For Type 3 Neovascularization/Retinal Angiomatous Proliferation." Retina, 29(10):1424-31 (2009)		
	36	ENGELBERT, "Long-Term Follow-Up For Type 1 (Subretinal Pigment Epithelium) Neovascularization Using A Modified 'Treat And Extend' Dosing Regiment Of Intravitreal Antivascular Endothelial Growth Factor Therapy." Retina, 30(9):1368-75 (2010)		
	37	ENGELBERT, "The 'Treat and Extend' Dosing Regimen of Intravitreal Anti-Vascular Endothelial Growth Factor Therapy for Neovascular Age-Related Macular Degeneration." Ophthalmology Management, Issue 42, (June 2010) available at http://www.visioncareprofessional.com/emails/amdupdate/index.asp?issue=42		
	38	EREMINA et al., "Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases" Journal of Clinical Investigation (March 2003) 111(5):707-716		
	39	ERIKSSON et al., "Structure, Expression and Receptor-Binding Properties of Novel Vascular Endothelial Growth Factors" Vascular Growth Factors and Angiogenesis, Springer (1999) pp. 41-57		
	40	THE EYETECH STUDY GROUP, "Anti-Vascular Endothelial Growth Factor Therapy for Subfoveal Choroidal Neovascularization Secondary to Age-related Macular Degeneration" American Academy of Ophthalmology, 110(5):979-986 (May 2003)		
	41	Eylea®, Highlights of Prescribing Information, Revised 08/2018.		
	42	FERRARA, N. "Vascular Endothelial Growth Factor: Molecular and Biological Aspects" Advances in Organ Biology (1999) pp. 1-30		
	43	FERRARA et al., "Clinical applications of angiogenic growth factors and their inhibitors" Nature Medicine (December 1999) 5(12):1359-1364		
	44	FLYVBJERG et al., "Amelioration of Long-Term Renal Changes in Obese Type 2 Diabetic Mice by a Neutralizing Vascular Endothelial Growth Factor Antibody" Diabetes (October 2002) 51:3090-3094		
	45	FUNG, "An Optical Coherence Tomography-Guided, Variable Dosing Regiment with Intravitreal Ranibizumab (Lucentis) for Neovascular Age-related Macular Degeneration." Am J Ophthalmology, 143(4):566-83 (April 2007)		
	46	GALE, "Complementary and Coordinated Roles of the VEGFs and Angiopoietins during Normal and Pathologic Vascular Formation." Cold Spring Harbor Symposia on Quantitative Biology, Volume LXVIL, pp. 267-73 (2002)		
	47	GARCIA-QUINTANILLA, "Pharmacokinetics of Intravitreal Anti-VEGF Drugs in Age-Related Macular Degeneration." Pharmaceutics, 11:365 (2019)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	5	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	48	GOMEZ-MANZANO, "VEGF Trap induces antiglioma effect at different stages of disease." Neuro-Oncology, 10:940-945 (December 2008)		
	49	GRAGOUDAS, "Pegaptanib for Neovascular Age-Related Macular Degeneration." N Engl J Med, 351(27):2805-16, (December 30, 2004)		
	50	GUTIERREZ et al., "Intravitreal bevacizumab (Avastin) in the treatment of macular edema secondary to retinal vein occlusion" Clin. Ophthalmol., 2(4):787,791 (2008)		
	51	HALLER et al., "VEGF Trap-Eye In CRVO: Primary Endpoint Results of the Phase 3 COPERNICUS Study" ARVO Annual Meeting Abstract (April 2011)		
	52	HEIER et al., "CLEAR-IT 2: Phase 2, Randomized Controlled Dose and Interval-Ranging Study of Intravitreal VEFG Trap Eye in Patients with Neovascular Age-Related Macular Degeneration: Predictive Factors for Visual Acuity" ARVO Annual Meeting Abstract (April 2009)		
	53	HEIER et al., " rhuFab V2 (anti-VEGF Antibody) for Treatment of Exudative AMD" Symposium 8:Experimental and Emerging Treatments for Choroidal Neovascularization, 10 pp (2002)		
	54	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)		
	55	HEIER et al., "Intravitreal Aflibercept (VEGF Trap-Eye) in Wet Age-related macular Degeneration," Ophthalmology, 119:2537-2548 (2012)		
	56	HEIER, "Intravitreal Aflibercept for Diabetic Macular Edema: 148-Week Results from the VISTA and VIVID Studies." Ophthalmology, 123(11):2376-2385 (November 2016)		
	57	HEIER et al., "The 1-year Results of CLEAR-IT 2, a Phase 2 Study of Vascular Endothelial Growth Factor Trap-Eye Dosed As-needed After 12-week Fixed Dosing" Ophthalmology 2011;118:1098–1106 (June 2011)		
	58	HEIER et al., "The 1-year Results of CLEAR-IT 2, a Phase 2 Study of Vascular Endothelial Growth Factor Trap-Eye Dosed As-needed After 12-week Fixed Dosing: Erratum" Ophthalmology 2011;118:1700 (September 2011)		
	59	HO, "VEGF Trap-Eye in Wet AMD - CLEAR-IT 2: One-Year OCT and FA Outcomes" CLEAR-IT 2 Study Group, pp 1-24 (09/28/2008)		
	60	Ho et al., Slides entitled CLEAR IT 2 One-Year Key Results, Retina Society (2008)		
	61	HOLASH et al., "Vessel Cooption, Regression, and Growth in Tumors Mediated by Angiopoietins and VEGF" Science (June 18, 1999) 284(5422):1994-1998		
	62	HOLASH, "VEGF-Trap: A VEGF blocker with potent antitumor effects" PNAS 99(17)11393-11398 (8/20/2002)		
	63	HOLASH, "Inhibitors of growth factor receptors, signaling pathways and angiogenesis as therapeutic molecular agents." Cancer Metastasis 25:243-252 (2006)		
	64	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00320775 "Safety and Tolerability of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration" 70 pages, Latest version submitted June 8, 2011 on ClinicalTrials.gov (NCT00320775_2006-2011)		
	65	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00320775 "Safety and Tolerability of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration" 10 pages, Latest version submitted March 16, 2015 on ClinicalTrials.gov (NCT00320775_2015)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	6	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	66	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00320788 "Safety and Efficacy of Repeated Intravitreal Administration of Vascular Endothelial Growth Factor (VEGF) Trap in Patients With Wet Age-Related Macular Degeneration (AMD)" 71 pages, Latest version submitted December 1, 2011 on ClinicalTrials.gov (NCT00320788_2006-2011)		
	67	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00320788 "Safety and Efficacy of Repeated Intravitreal Administration of Vascular Endothelial Growth Factor (VEGF) Trap in Patients With Wet Age-Related Macular Degeneration (AMD)" 31 pages, Latest version submitted January 27, 2012 on ClinicalTrials.gov (NCT00320788_2012)		
	68	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00320814 "Phase 1 Study of VEGF Trap in Patients With Diabetic Macular Edema" 30 pages, Latest version submitted June 8, 2011 on ClinicalTrials.gov (NCT00320814_2006-2011)		
	69	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00509795 "Double-Masked Study of Efficacy and Safety of IVT VEGF Trap-Eye in Subjects With Wet AMD (VIEW 1)" 318 pages, Latest version submitted December 1, 2011 on ClinicalTrials.gov (NCT00509795_2007-2011)		
	70	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00509795 "Double-Masked Study of Efficacy and Safety of IVT VEGF Trap-Eye in Subjects With Wet AMD (VIEW 1)" 200 pages, Latest version submitted December 20, 2012 on ClinicalTrials.gov (NCT00509795_2012)		
	71	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00527423 "Randomized, Single-Masked, Long-Term, Safety and Tolerability Study of VEGF Trap-Eye in AMD" 64 pages, Latest version submitted November 1, 2011 on ClinicalTrials.gov (NCT00527423_2007-2011)		
	72	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00527423 "Randomized, Single-Masked, Long-Term, Safety and Tolerability Study of VEGF Trap-Eye in AMD" 42 pages, Latest version submitted June 10, 2013 on ClinicalTrials.gov (NCT00527423_2012-2013)		
	73	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00637377 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) (VIEW 2)" 667 pages, Latest version submitted December 16, 2011 on ClinicalTrials.gov (NCT00637377_2008-2011)		
	74	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00637377 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) (VIEW 2)" 289 pages, Latest version submitted November 28, 2014 on ClinicalTrials.gov (NCT00637377_2012-2014)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	7	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	75	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00789477 "DME And VEGF Trap-Eye [Intravitreal Aflibercept Injection (IAI;EYLEA®;BAY86-5321)] INvestigation of Clinical Impact (DA VINCI)" 135 pages, Latest version submitted May 2, 2011 on ClinicalTrials.gov (NCT00789477_2008-2011)		
	76	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00789477 "DME And VEGF Trap-Eye [Intravitreal Aflibercept Injection (IAI;EYLEA®;BAY86-5321)] INvestigation of Clinical Impact (DA VINCI)" 53 pages, Latest version submitted August 28, 2014 on ClinicalTrials.gov (NCT00789477_2013-2014)		
	77	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00943072 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)" 98 pages, Latest version submitted May 9, 2011 on ClinicalTrials.gov (NCT00943072_2009-2011)		
	78	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00943072 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)" 64 pages, Latest version submitted April 16, 2013 on ClinicalTrials.gov (NCT00943072_2012-2013)		
	79	Information from ClinicalTrials.gov archive View of NCT00637377 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) (VIEW 2)" *ClinicalTrials.gov.* Web. (2010-11-30).		
	80	Information from ClinicalTrials.gov archive on the VIEW 2 study (NCT00637377) "VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD (VIEW 2)" version available (updated on 17 March 2008)		
	81	Information from ClinicalTrials.gov archive on the view of NCT00509795 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD)" (12-01-2009)		
	82	Information from ClinicalTrials.gov archive on the view of NCT00789477 "DME and VEGF Trap-Eye: Investigation of Clinical Impact" (11-18-2010)		
	83	Information from ClinicalTrials.gov archive on the view of NCT00509795 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD)" (01-07-2011)		
	84	Information from ClinicalTrials.gov archive on the view of NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 7 pages, first posted 11/13/2009; results first posted 11/22/2012; last update posted 11/3/14; printed 12/4/19 (https://clinicaltrials.gov/ct2/show/study/NCT01012973) (NOTE: May correspond to "Vascular Endothelial Growth Factor Trap‐ Eye Investigation of Efficacy and Safety in Central Retinal Vein Occlusion title, 8 pages, 11/12/2009, US **[Cited in Third Party Observations filed in parent application USSN 16/055,847 for which a copy is unavailable on PAIR]"** which was cited in the Third Party Observations dated 05/01/19)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	8	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	85	KAISER, "Vascular endothelial growth factor Trap-Eye for diabetic macular oedema." Br. J. Ophthalmol, 93(2):135-36 (February 2009)		
	86	KARIA, Niral, "Retinal vein occlusion: pathophysiology and treatment options" Clinical Ophthalmology, 4:809-816 (2010)		
	87	KOROBELNIK et al., "Intravitreal Aflibercept Injection for Macular Edema Resulting from Central Retinal Vein Occlusion" American Academy of Ophthalmology (2014) 121(1):202-208		
	88	KOROBELNIK, "Intravitreal Aflibercept for Diabetic Macular Edema." Ophthalmology, 121(11):2247-54 (November 2014)		
	89	KUO, "Comparative evaluation of the antitumor activity of antiangiogenic proteins delivered by gene transfer" PNAS 98(8):4605-4610 (04/10/2001)		
	90	KRZYSTOLIK et al., "Prevention of Experimental Choroidal NEovascularization With Intravitreal Anti-Vascular Endothelial Growth Factor Antibody Fragment" Arch Ophthamol., 120:338-346 (Mar. 2002)		
	91	LALWANI, "All About PrONTO: Study Yielded Good Results in AMD With Treatment Guided by OCT." Retina Today (May 2007)		
	92	LALWANI, 'A Variable-dosing Regimen with Intravitreal Ranibizumab for Neovascular Age-related Macular Degeneration: Year 2 of the PrONTO Study." Am J Ophthalmology, 148(1):43-58 (July 2009)		
	93	LEVINE, "Macular Hemorrhage In Neovascular Age-Related Macular Degeneration After Stabilization With Antiangiogenic Therapy." Retina, 29(8):1074-79 (2009)		
	94	Lucentis Label Title, 7 pages, 30/06/2010 [**Cited in Third Party Observations filed in parent application USSN 16/055,847 for which a copy is unavailable on PAIR**]		
	95	MAJOR et al., "DA VINCI: DME and VEGF Trap-Eye: Investigation of Clinical Impact: Phase 2 Study in Patients with Diabetic Macular Edema (DME)" ARVO Annual Meeting Abstract (April 2010)		
	96	MARGOLIS, "Hemorrhagic Recurrence Of Neovascular Age-Related Macular Degeneration Not Predicted By Spectral Domain Optical Coherence Tomography." Retinal Cases & Brief Reports, 4:1-4 (2010)		
	97	MASSIN, "Safety and Efficacy of Ranibizumab in Diabetic Macular Edema (RESOLVE Study*)." Diabetes Care, 33(11):2399-405 (November 2010)		
	98	MITCHELL, "The RESTORE Study, Ranibizumab Monotherapy or Combined with Laser versus Laser Monotherapy for Diabetic Macular Edema." Ophthalmology, 188(4):615-25 (April 2011)		
	99	MITCHELL, Edith P. "Targeted Therapy for Metastatic Colorectal Cancer: Role of Aflibercept" Clinical Colorectal Cancer (2013) 12(2):73-85		
	100	MITRA et al., "Review of anti-vascular endothelial growth factor therapy in macular edema secondary to central retinal vein occlusions" Expert Review in Ophthalmol, Taylor & Francis, GB 6(6):623-629 (January 2011)		
	101	MOUSA AND MOUSA, "Current Status of Vascular Endothelial Growth Factor Inhibition in Age-Related Macular Degeneration" Biodrugs; 24(3); 183-194 (2010)		
	102	N/A "Materials from June 2011 FDA Committee Mtg" (06/17/2011)		
	103	N/A "Materials from Dec 2011 FDA Committee Mtg"(12/01/2011)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	9	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	104	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)		
	105	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, 113(9):1522e1-1522e14 (Sept 2006) (epub July 28,2006)		
	106	NGUYEN et al., "Randomized, Double-masked, Active-controlled Phase 3 Trial of the Efficacy and Safety of Intravitreal VEGF Trap-Eye in Wet AMD: One-year Results of the VIEW 1 Study" ARVO Annual Meeting Abstract (April 2011)		
	107	NGUYEN, "Ranibizumab for Diabetic Macular Edema, Results from 2 Phase III Randomized Trials: RISE and RIDE." Ophthalmology, 119(4):789-801 (April 2012)		
	108	NGUYEN et al., "Results of a Phase I, Dose-Escalation, Safety, Tolerability, and Bioactivity Study of Intravitreous VEGF Trap in Patients with Neovascular Age-Related Macular Degeneration" ARVO Annual Meeting Abstract (May 2006)		
	109	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, 2003)		
	110	NOGUERA-TROISE et al., "Blockade of D114 inhibits tumour growth by promoting non-productive angiogenesis" Nature (December 2006) 444:1032-1037		
	111	OHR, "Aflibercept in wet age-related macular degeneration: a perspective review" Ther. Adv. Chronic Dis., 3(4):153-161 (2012)		
	112	OLIVERA et al., "VEGF Trap R1R2 suppresses experimental corneal angiogenesis" European Journal of Ophthalmology, 20(1):48-54 (January 1, 2010)		
	113	PAI et al., "Current concepts in intravitreal drug therapy for diabetic retinopathy" Saudi Journal of Ophthalmology 24(4):143-149 (June 30, 2010)		
	114	PAPADPPOULOS, "Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab" Angiogenesis, 15:171-185 (2012)		
	115	Regeneron SEC Form 10-K (February 27, 2008)		
	116	Regeneron SEC Form 10-K (February 26, 2009)		
	117	Regeneron SEC Form 10-K (February 17, 2011)		
	118	Regeneron SEC Form 10-Q (May 8, 2006)		
	119	Regeneron SEC Form 10-Q (August 8, 2006)		
	120	Regeneron SEC Form 10-Q (November 6, 2006)		
	121	Regeneron SEC Form 10-Q (May 4, 2007)		
	122	Regeneron SEC Form 10-Q (August 3, 2007)		
	123	Regeneron SEC Form 10-Q (April 30, 2009)		
	124	Regeneron SEC Form 10-Q (November 3, 2009)		
	125	Regeneron SEC Form 10-Q (April 29, 2010)		
	126	Regeneron SEC Form 10-Q (July 28, 2010)		
	127	Regeneron SEC Form 10-Q (October 28, 2010)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	10	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	128	Regeneron SEC Form 10-Q (May 3, 2011)		
	129	Regeneron SEC Form 10-Q (July 28, 2011)		
	130	Regeneron SEC Form 10-Q (October 27, 2011)		
	131	Regeneron SEC Form 8-K Exhibit: "Press Release of Regeneron Pharmaceuticals, Inc. dated May 1, 2006" (May 2, 2006)		
	132	Regeneron SEC Form 8-K Exhibit: "Press Release of Regeneron Pharmaceuticals, Inc. dated May 3, 2006" (May 5, 2006)		
	133	Regeneron SEC Form 8-K Exhibit: "Slides presented at the Company's 2006 Annual Meeting of Shareholders held on June 9, 2006" (June 9, 2006)		
	134	Regeneron SEC Form 8-K Exhibit: "Press Release dated May 2, 2007" (May 3, 2007)		
	135	Regeneron SEC Form 8-K Exhibit: "Overheads for presentation at Regeneron's Annual Meeting of Shareholders to be held on June 8, 2007" (June 8, 2007)		
	136	Regeneron SEC Form 8-K Exhibit: "Press Release dated October 1, 2007" (October 1, 2007)		
	137	Regeneron SEC Form 8-K Exhibit: "Press Release dated November 6, 2007" (November 6, 2007)		
	138	Regeneron SEC Form 8-K Exhibit: "Press Release dated May 1, 2008" (May 2, 2008)		
	139	Regeneron SEC Form 8-K Exhibit: "Press Release dated November 4, 2008" (November 4, 2008)		
	140	Regeneron SEC Form 8-K Exhibit: "99(a) Slides that Regeneron Pharmaceuticals, Inc. intends to use in conjunction with meetings with investors at the J.P. Morgan 27th Annual Healthcare Conference in San Francisco on January 12-15, 2009." (January 9, 2009)		
	141	Regeneron SEC Form 8-K Exhibit: "Press Release dated April 30, 2009" (May 1, 2009)		
	142	Regeneron SEC Form 8-K Exhibit: "Press Release dated November 3, 2009." (November 4, 2009)		
	143	Regeneron SEC Form 8-K Exhibit: "Press Release Reporting Positive Results for VEGF Trap-Eye in Phase 3 Study in Central Retinal Vein Occlusion (CRVO) and in Phase 2 Study in Diabetic Macular Edema (DME) dated December 20, 2010." (December 20, 2010)		
	144	Regeneron SEC Form 8-K Exhibit: "Press Release dated February 17, 2011" (February 18, 2011)		
	145	Regeneron SEC Form 8-K Exhibit: "Press Release Reporting Positive Results for VEGF Trap-Eye in Second Phase 3 Study in Central Retinal Vein Occlusion, dated April 27, 2011" (April 27, 2011)		
	146	Regeneron SEC Form 8-K Exhibit: "Press Release dated May 3, 2011." (May 3, 2011)		
	147	Regeneron SEC Form 8-K Exhibit: "Press Release, dated June 17, 2011, Announcing that EYLEA™ (aflibercept ophthalmic solution) Received Unanimous Recommendation for Approval for Treatment of Wet AMD from FDA Advisory Committee." (June 21, 2011)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	11	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	148	Regeneron SEC Form 8-K Exhibit: "Presentation entitled VEGF Trap-Eye in CRVO: 1-year Results of the Phase 3 COPERNICUS Study" (August 22, 2011)		
	149	Regeneron SEC Form 8-K Exhibit: "Press Release Announcing FDA Approval of EYLEA™ (aflibercept) Injection for the Treatment of Wet Age-Related Macular Degeneration, dated November 18, 2011" (November 21, 2011)		
	150	Regeneron Press Release "Positive Interim Phase 2 Data Reported For VEGF Trap-Eye In Age-Related Macular Degeneration" (March 27, 2007)		
	151	Regeneron Press Release "VEGF TRAP-Eye Phase 2 Wet AMD Results Reported At Arvo Annual Meeting" (May 9, 2007)		
	152	Regeneron Press Release "Regeneron Reports Second Quarter Financial And Operating Results" (August 1, 2007)		
	153	Regeneron Pharmaceuticals, Inc., "Regeneron and Bayer Healthcare Initiate Phase 3 Global Development Program for VEGF Trap-Eye In Wet Age-Related Macular Degeneration (AMD)" (August 2, 2007)		
	154	Regeneron Pharmaceuticals, Inc. FORM 10-Q, published on 7 November 2007 for the period ending 30 September 2007		
	155	Regeneron Press Release "Regeneron Announces Positive Primary Endpoint Results From A Phase 2 Study Of VEGF Trap-Eye In Age-Related Macular Degeneration" (October 1, 2007)		
	156	Regeneron Press Release "Regeneron Reports Fourth Quarter And Full Year 2007 Financial And Operating Results" (February 27, 2008)		
	157	Regeneron Pharmaceuticals, Inc., "Regeneron and Bayer HealthCare Announce Encouraging 32-Week Follow-up Results from a Phase 2 Study of VEGF Trap-Eye in Age-Related Macular Degeneration" (April 28, 2008)		
	158	Regeneron, Press release "Regeneron Reports First Quarter 2008 Financial and Operating Results", May 1, 2008.		
	159	Regeneron Press Release, "Bayer and Regeneron Dose First Patient in Second Phase 3 Study for VEGF Trap-Eye in Wet Age-Related Macular Degeneration." May 8, 2008		
	160	Regeneron Pharmaceuticals Inc., "CLEAR-IT-2: Interim Results Of The Phase II, Randomized, Controlled Dose-and Interval-ranging Study Of Repeated Intravitreal VEGF Trap Administration In Patients With Neovascular Age-related Macular Degeneration (AMD)" poster presented at the 2007 Association for Research in Vision and Ophthalmology meeting in Ft. Lauderdale, Florida (May 2007)		
	161	Regeneron Pharmaceuticals Inc., "An Exploratory Study of the Safety, Tolerability and Biological Effect of a Single Intravitreal Administration of VEGF Trap in Patients with Diabetic Macular Edema" poster presented at the 2007 Association for Research in Vision and Ophthalmology meeting in Ft. Lauderdale, Florida (May 2007)		
	162	Regeneron Pharmaceuticals Inc., "Optical Coherence Tomography Outcomes of a Phase 1, Dose-Escalation, Safety, Tolerability, and Bioactivity Study of Intravitreal VEGF Trap in Patients with Neovascular Age-Related Macular Degeneration: The CLEAR-IT 1 Study" poster presented at the 2007 Association for Research in Vision and Ophthalmology meeting in Ft. Lauderdale, Florida (May 2007)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	12	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	163	Regeneron Pharmaceuticals, Inc., "Regeneron and Bayer HealthCare Announce VEGF Trap-Eye Achieved Durable Improvement in Vision over 52 Weeks in a Phase 2 Study in Patients with Age-related Macular Degeneration" (August 19, 2008)		
	164	Regeneron Pharmaceuticals Inc., " VIEW 1 Vascular Endothelial Growth Factor (VEGF) Trap-Eye 1-Year Results: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) " presented at Bascom Palmer Eye Institute's Angiogenesis, Exudation and Degeneration 2011 meeting in Miami, Florida (February 12, 2011)		
	165	Regeneron Pharmaceuticals Inc., " VIEW 2 Vascular Endothelial Growth Factor (VEGF) Trap-Eye 1-Year Results: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) " presented at Bascom Palmer Eye Institute's Angiogenesis, Exudation and Degeneration 2011 meeting in Miami, Florida (February 12, 2011)		
	166	Regeneron Pharmaceuticals Inc., "VEGF Trap-Eye CLEAR-IT 2 Final Primary Endpoint Results" presented at the 2007 Retina Society Conference in Boston, Massachusetts (September 30, 2007)		
	167	Regeneron Pharmaceuticals Inc., "VEGF Trap-Eye Final Phase 2 Results in Age-related Macular Degeneration Presented at 2008 Retina Society Meeting" (September 28, 2008) (XP-002770952)		
	168	Regeneron 2008 Annual Report		
	169	Regeneron Pharmaceuticals, Inc. "Regeneron Reports Full Year and Fourth Quarter 2008 Financial and Operating Results" (February 26, 2009)		
	170	Regeneron Pharmaceuticals, Inc. "Bayer and Regeneron Extend Development Program for VEGF Trap-Eye to Include Central Retinal Vein Occlusion" (April 30, 2009)		
	171	Regeneron Press Release "First Patient Enrolled In Regeneron And Bayer Healthcare VEGF Trap-Eye Phase 3 Program In Central Retinal Vein Occlusion" (July 23, 2009)		
	172	Regeneron Press Release "Enrollment Completed in Regeneron and Bayer HealthCare Phase 3 Studies of VEGF Trap-Eye in Neovascular Age-Related Macular Degeneration (Wet AMD)" September 14, 2009		
	173	Regeneron 2009 Annual Report and 10-K		
	174	Regeneron Press Release, "VEGF Trap-Eye Shows Positive Results in a Phase 2 Study in Patients With Diabetic Macular Edema." February 18, 2010		
	175	Regeneron Press Release "Regeneron Schedules November 22, 2010 Teleconference And Webcast To Discuss Results Of Two Phase 3 Studies With VEGF Trap-Eye In Wet Age-Related Macular Degeneration" (November 19, 2010)		
	176	Regeneron Press Release "Bayer and Regeneron Report Positive Top-Line Results of Two Phase 3 Studies with VEGF Trap-Eye in Wet Age-related Macular Degeneration" November 22, 2010		
	177	Regeneron Press Release "Regeneron and Bayer Report Positive Results for VEGF Trap-Eye in Phase 3 Study in Central Retinal Vein Occlusion (CRVO) and in Phase 2 Study in Diabetic Macular Edema (DME)" December 20, 2010		
	178	Regeneron 2010 Annual Report and 10-K		
	179	Regeneron Press Release "Regeneron And Bayer Start Phase 3 Trial To Extend Ophthalmology Research & Development Program For VEGF Trap-Eye In Asia" (January 18, 2011)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	13	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	180	Regeneron Press Release "Regeneron To Webcast Investor Briefing On VEGF Trap-Eye Clinical Program On Sunday, February 13th At 9 Am Et" (February 9, 2011)		
	181	Regeneron Press Release "Regeneron Submits Biologics License Application To FDA For VEGF Trap-Eye For Treatment Of Wet Age-Related Macular Degeneration" (February 22, 2011)		
	182	Regeneron Press Release "Regeneron And Bayer Announce Start Of Phase 3 Clinical Program In Diabetic Macular Edema" (April 8, 2011)		
	183	Regeneron Pharmaceuticals, Inc., "FDA Grants Priority Review for VEGF Trap-Eye for the Treatment of Wet Age-Related Macular Degeneration" (April 18, 2011)		
	184	Regeneron Press Release "VEGF Trap-Eye Submitted for EU Marketing Authorization for Treatment of Wet Age-Related Macular Degeneration (June 7, 2011)		
	185	Regeneron Pharmaceuticals, Inc., "Regeneron Announces EYLEA™ (aflibercept ophthalmic solution) Receives Unanimous Recommendation for Approval for Treatment of Wet AMD from FDA Advisory Committee" (June 17, 2011)		
	186	Regeneron Press Release "Regeneron Announces Clinical Presentations at ASRS 2011 Annual Meeting" (August 17, 2011)		
	187	Regeneron Pharmaceuticals, Inc., "Regeneron Announces FDA Approval of EYLEA™ (aflibercept) Injection for the Treatment of Wet Age-Related Macular Degeneration: CORRECTED (November 18, 2011)		
	188	Regeneron Pharmaceuticals, Inc., "Regeneron and Bayer Initiate Phase 3 Clinical Program for the Treatment of Wet Age-Related Macular Degeneration in China" (November 28, 2011)		
	189	Regeneron Pharmaceuticals, Inc., "Two Year Results of Phase 3 Studies with EYLEA™ (aflibercept) Injection in wet AMD Show Sustained Improvement in Visual Acuity" (December 5, 2011)		
	190	REGILLO et al., "Randomized, Double-Masked, Sham-Controlled Trial of Ranibizumab for Neovascular Age-related Macular Degeneration: OIER Study Year 1" American Journal of Ophthalmology, 145(2):239-248 (2008)		
	191	ROSENFELD, "Ranibizumab for Neovascular Age-Related Macular Degeneration." N Engl J Med, 355(14):1419-31 (October 5, 2006)		
	192	ROSENFELD, "Lessons Learned From Avastin and OCT-The Great, the Good, the Bad, and the Ugly: The LXXV Edward Jackson Memorial Lecture." Am. J. Ophthalmology, 204:26-45 (August 2019)		
	193	RUDGE et al., "VEGF Trap complex formation measures production rates of VEGF, providing a biomarker for predicting efficacious angiogenic blockade" PNAS (November 20, 2007) 104(47):18363-18370		
	194	RUDGE et al. "Clinical Development of VEGF Trap" In: Figg W.D., Folkman J. (eds) Angiogenesis (2008)		
	195	SCHMIDT-ERFURTH, "Efficacy and Safety of Monthly versus Quarterly Ranibizumab Treatment in Neovascular Age-related Macular Degeneration: The EXCIE Study" Ophthalmology, 118(5)831-839 (2010)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	14	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	196	SCHMIDT-ERFURTH et al. "Primary Results of an International Phase III Study Using Intravitreal VEGF Trap-Eye Compared to Ranibizumab in Patients with Wet AMD (VIEW 2)" ARVO Annual Meeting Abstract (April 2011)		
	197	SCHMIDT-ERFURTH, "Three-Year Outcomes of Individualized Ranibizumab Treatment in Patients with Diabetic Macular Edema." Ophthalmology, 121(5):1045-53,(May 2014)		
	198	SCHMIDT-ERFURTH et al., "Intravitreal Aflibercept Injection for Neovascular Age-related Macular Degeneration" Ophthalmology (2014) 121:193-201		
	199	SCHNICHELS, "Comparative toxicity and proliferation testing of aflibercept, bevacizumab and ranibizumab on different ocular cells." Br. J. Ophthalmol., 97:917-923 (2013)		
	200	SEMERARO et al., "Aflibercept in wet AMD: specific role and optimal use" Drug Design, Development and Therapy (August 2, 2013) 7:711-722		
	201	SHARMA and S. AND KAISER, P. K., Update on VEGF TRAP-Eye Clinical Trials and Retinal. Physician, pp. 1-6 (Nov/Dec 2010) <URL: https://www.retinalphysician.com/issues/2010/nov-dec/update-on-vegf-trap-eye-clinical-trials>		
	202	SIMO AND HERNANDEZ, "Advances in Medical Treatment of Diabetic Retinopathy" Diabetes Care, 32(8):1556-1562 (August 2009)		
	203	SLAKTER et al., "Influence of Baseline Angiographic Classification on Outcomes in the CLEAR-IT 2 Phase 2 Study of Intravitreal VEGF Trap-Eye in Neovascular Age-Related Macular Degeneration" ARVO Annual Meeting Abstract (April 2010)		
	204	SLAKTER et al., "A Phase 2, Randomized, Controlled Dose-and Interval-Ranging Study of Intravitreal VEGF Trap-Eye in Patients with Neovascular Age-Related Macular Degeneration: Optical Coherence Tomography (OCT) and Fluorescein Angiography (FA) Outcomes at 1 Year" ARVO Annual Meeting Abstract (April 2009)		
	205	Slides for the 2008 Retina Society Meeting "VEGF Trap-Eye in Wet AMD CLEAR-IT 2: Summary of One-Year Key Results", September 28, 2008.		
	206	SPAIDE, "Ranibizumab According to Need: A Treatment for Age-related Macular Degeneration." Am J Ophthalmology, 143(4):679-680 (April 2007)		
	207	STEWART, "The expanding role of vascular endothelial growth factor inhibitors in opthamology" Mayo Clin Proc. 87(1):77-88 (January 2012)		
	208	STEWART et al., "Predicted biological activity of intravitreal VEGF Trap" British Journal of Ophthalmology, 92(5):667-668 (2008)		
	209	STEWART, "Aflibercept" Nature Reviews: Drug Discovery 11:269-270 (04/01/2012)		
	210	TANNOCK et al., "Aflibercept versus placebo in combination with docetaxel and prednisone for treatment of men with metastatic castration-resistant prostate cancer (VENICE): a phase 3, double-blind randomized trial" Lancet Oncol (2013) 14:760-768		
	211	THOMAS REUTERS INTEGRITY "VEGF Trap-Eye final phase II results in age-related macular degeneration presented at 2008 Retina Society Meeting" (September 28, 2008)		
	212	THURSTON, Gavin "Complementary actions of VEGF and Angiopoietin-1 on blood vessel growth and leakage" J. Anat. (2002) 200:575-580		
	213	THURSTON, "Vascular endothelial growth factor and other signaling pathways in developmental and pathologic angiogenesis." International Journal of Hematology, 80:7-20 (2004)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	15	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	214	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 38 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_01182013_27424.1)		
	215	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_01252011_27433.1)		
	216	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 11 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_01262012_27428.1)		
	217	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 38 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_01302013_27423.1)		
	218	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_02092010_27442.1)		
	219	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 11 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_02202012_27427.1)		
	220	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_03162010_27441.1)		
	221	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_04082011_27432.1)		
	222	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_04162010_27440.1)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	16	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	223	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_06232011_27431.1)		
	224	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_07222010_27439.1)		
	225	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_08252010_27438.1)		
	226	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_08262010_27437.1)		
	227	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_09082010_27436.1)		
	228	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_09192011_27430.1)		
	229	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_10042010_27435.1)		
	230	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 38 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_10232012_27426.1)		
	231	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 38 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_10272013_27422.1)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	17	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	232	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_11012010_27434.1)		
	233	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_11132009_27444.1)		
	234	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_11292011_27429.1)		
	235	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 38 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_12182012_27425.1)		
	236	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_12212010_27443.1)		
	237	Vascular Endothelial Growth Factor Trap‐ Eye Investigation of Efficacy and Safety in Central Retinal Vein Occlusion title, 8 pages, 11/12/2009, US **[Cited in Third Party Observations filed in parent application USSN 16/055,847 for which a copy is unavailable on PAIR] NOTE:** May correspond to **"**Information from ClinicalTrials.gov archive on the view of NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 7 pages, first posted 11/13/2009; results first posted 11/22/2012; last update posted 11/3/14; printed 12/4/19 (https://clinicaltrials.gov/ct2/show/study/NCT01012973)" cited by the Examiner in the Office Action dated 12/10/19 in USSN 16/055,847		
	238	WACHSBERGER, "VEGF trap in combination with radiotherapy improves tumor control in u87 glioblastoma." Int. J. Radiation Oncology Biol Phys. 67(5):1526-1537 (2007)		
	239	WHO Drug Information, "International Nonproprietary Names for Pharmaceutical Substances (INN)" 20(2):115-119 (2006)		
	240	WOLFSON, "Regeneron Focuses on Age-Related Macular Degeneration." Chemistry & Biology 15:303-304 (April 2008)		
	241	XIA et al., "Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis" Blood (July 1, 2003) 102(1):161-168		
	242	YANCOPOULOS, "Vascular-specific growth factors and blood vessel formation." Nature 407:242-48 (September 14, 2000)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	18	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	243	YANCOPOULOS, "Clinical Application of Therapies Targeting VEGF." Cell 143:13-16 (October 1, 2010)		
	244	YUNG, "Moving Toward the Next Steps in Angiogenesis Therapy?" Society for Neuro-Oncology, 10:939 (2008)		

Examiner Signature		Date Considered	

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

REMARKS UNDER 37 CFR § 1.115

Formal Matters

Claims 21-72 are pending after entry of the amendments set forth herein.

Original claims 1-20 were previously canceled without prejudice.

Claims 21-72 are added here.

Support for new claims 21-72 can be found in originally pending now canceled claims 1-20, and throughout the specification.

No new matter has been added.

SEQUENCE LISTING

Applicants submit herewith the attached Sequence Listing in .txt format. As set out in MPEP §2422.03(a), the Office has advised that if the sequence listing text file submitted via EFS-Web complies with the requirements of 37 CFR 1.824(a)(2)-(6) and (b) (i.e., is a compliant sequence listing ASCII text file), the text file will serve as both the paper copy required by 37 CFR 1.821(c) and the computer readable form (CRF) required by 37 CFR 1.821(e). Further, per MPEP §2422.03(a), neither (1) a second copy of the sequence listing in a PDF file; nor (2) a statement under 37 CFR 1.821(f) (indicating that the paper copy and CRF copy of the sequence listing are identical) should be submitted.

The Sequence Listing was prepared with the software FASTSEQ for Windows version 4.0, and conforms to the Patent Office guidelines. Applicant respectfully submits that the subject application is in adherence to 37 CFR §§ 1.821-1.825. I hereby certify that the enclosed submission includes no new matter.

Applicants respectfully submit that the present patent application is now in compliance with 37 CFR §§ 1.821-1.825.

STATEMENT UNDER 37 C.F.R. §§1.56 AND 1.2

Applicants hereby advise the Examiner of the status of a co-pending application in compliance with the Applicant's duty to disclose under 37 C.F.R. §§1.56 and 1.2 (see also MPEP §2001.06(b)) as discussed in *McKesson Info. Soln. Inc., v. Bridge Medical Inc.*, 487 F.3d 897; 82 USPQ2d 1865 (Fed. Cir. 2007).

The Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 13/940,370, filed July 12, 2013 which issued on February 9, 2016 as U.S. Patent 9,254,338.

The Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 14/972,560, filed December 17, 2015 which issued on June 6, 2017 as U.S. Patent No. 9,669,069.

The Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 15/471,506, filed March 28, 2017 which issued on November 20, 2018 as U.S. Patent No. 10,130,681.

The Applicant wishes to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/055,847, filed August 6, 2018 for which a Notice of Allowance was mailed on July 22, 2020 and the Issue Fee was paid on October 8, 2020.

The Applicant wishes to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/159,282, filed October 12, 2018 for which a Notice of Allowance was mailed on July 22, 2020 and the Issue Fee was paid on October 8, 2020.

The Applicant wishes to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/397,267, filed April 29, 2019 for which an Office Action was mailed on May 12, 2020.

These documents are available on PAIR, and thus are not provided with this communication. Please inform the undersigned if there is any difficulty in obtaining the documents from PAIR.

CONCLUSION

Applicant submits 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, please 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-008CIPCON6.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 16 October 2020

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic, Reg. No. 28,807

Bozicevic, Field & Francis LLP
201 Redwood Shores Parkway, Suite 200
Redwood City, California 94065
Telephone: (650) 327-3400
Direct: (650) 833-7735
Facsimile: (650) 327-3231

AMENDMENTS TO THE CLAIMS

1. - 20. (**Canceled**)

21. (**New**) A method of treating macular edema following retinal vein occlusion in a patient in need thereof comprising administering 2 mg aflibercept to the patient by intravitreal injection approximately once every 4 weeks, wherein the patient achieves a gain in visual acuity following treatment.

22. (**New**) The method of claim 21 wherein the patient has macular edema following central retinal vein occlusion.

23. (**New**) The method of claim 21 wherein the patient achieves a gain in Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

24. (**New**) The method of claim 21 wherein the patient achieves a gain in visual acuity at 24 weeks following the initial dose.

25. (**New**) The method of claim 24 wherein the patient gains at least 5 letters Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score at 24 weeks following the initial dose.

26. (**New**) The method of claim 24 wherein the patient gains at least 10 letters Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score at 24 weeks following the initial dose.

27. (**New**) The method of claim 24 wherein the patient gains at least 15 letters Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score at 24 weeks following the initial dose.

28. (**New**) The method of claim 21 wherein the patient achieves a gain in visual acuity at 52 weeks following the initial dose.

29. (**New**) The method of claim 28 wherein the patient gains at least 5 letters Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score at 52 weeks following the initial dose.

30. (**New**) The method of claim 28 wherein the patient gains at least 10 letters Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score at 52 weeks following the initial dose.

31. (**New**) The method of claim 28 wherein the patient gains at least 15 letters Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score at 52 weeks following the initial dose.

32. (**New**) The method of claim 21 wherein exclusion criteria for the patient include (1) active intraocular inflammation; or (2) active ocular or periocular infection.

33. (**New**) The method of claim 32 wherein the patient achieves a gain in Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

34. (**New**) The method of claim 31 wherein the patient achieves a gain in visual acuity at 24 weeks following the initial dose.

35. (**New**) The method of claim 34 wherein the patient gains at least 5 letters Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score at 24 weeks following the initial dose.

36. (**New**) The method of claim 34 wherein the patient gains at least 10 letters Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score at 24 weeks following the initial dose.

37. (**New**) The method of claim 34 wherein the patient gains at least 15 letters Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score at 24 weeks following the initial dose.

38. (**New**) The method of claim 32 wherein the patient achieves a gain in visual acuity at 52 weeks following the initial dose.

39. (**New**) The method of claim 38 wherein the patient gains at least 5 letters Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score at 52 weeks following the initial dose.

40. (**New**) The method of claim 38 wherein the patient gains at least 10 letters Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score at 52 weeks following the initial dose.

41. (**New**) The method of claim 38 wherein the patient gains at least 15 letters Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score at 52 weeks following the initial dose.

42. (**New**) A method of treating macular edema following retinal vein occlusion in a patient in need thereof comprising administering 2 mg aflibercept to the patient by intravitreal injection approximately once every 4 weeks, wherein the patient achieves a reduction in central retinal thickness as measured by optical coherence tomography (OCT) following treatment.

43. (**New**) The method of claim 42 wherein the patient has macular edema following central retinal vein occlusion.

44. (**New**) The method of claim 42 wherein the patient achieves a reduction in central retinal thickness at 24 weeks following the initial dose.

45. (**New**) The method of claim 44 wherein the patient achieves a reduction in central retinal thickness of at least 400 µm at 24 weeks following the initial dose.

46. (**New**) The method of claim 42 wherein the patient achieves a reduction in central retinal thickness at 52 weeks following the initial dose.

47. (**New**) The method of claim 46 wherein the patient achieves a reduction in central retinal thickness of at least 400 µm at 52 weeks following the initial dose.

48. (**New**) The method of claim 42 wherein exclusion criteria for the patient include (1) active intraocular inflammation; or (2) active ocular or periocular infection.

49. (**New**) The method of claim 48 wherein the patient achieves a reduction in central retinal thickness at 24 weeks following the initial dose.

50. (**New**) The method of claim 49 wherein the patient achieves a reduction in central retinal thickness of at least 400 µm at 24 weeks following the initial dose.

51. (**New**) The method of claim 48 wherein the patient achieves a reduction in central retinal thickness at 52 weeks following the initial dose.

52. (**New**) The method of claim 51 wherein the patient achieves a reduction in central retinal thickness of at least 400 µm at 52 weeks following the initial dose.

53. (**New**) A method of treating macular edema following retinal vein occlusion in a patient in need thereof comprising administering an initial dose of 2 mg aflibercept to the patient by intravitreal injection and subsequently administering one or more additional doses of 2 mg aflibercept to the patient by intravitreal injection on an as needed/pro re nata (PRN) basis, based on visual and/or anatomical outcomes as assessed by a physician or other qualified medical professional.

54. (**New**) The method of claim 53 wherein the patient achieves a gain in visual acuity following treatment.

55. (**New**) The method of claim 54 wherein the patient achieves a gain in Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

56. (**New**) The method of claim 53 wherein the patient achieves a gain in visual acuity at 12 weeks following the initial dose.

57. (**New**) The method of claim 56 wherein the patient achieves a gain in Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

58. (**New**) The method of claim 53 wherein the patient achieves a gain in visual acuity at 24 weeks following the initial dose.

59. (**New**) The method of claim 58 wherein the patient achieves a gain in Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

60. (**New**) The method of claim 53 wherein the patient achieves a reduction in central retinal thickness as measured by optical coherence tomography (OCT) following treatment.

61. (**New**) The method of claim 60 wherein the patient achieves a reduction in central retinal thickness at 12 weeks following the initial dose.

62. (**New**) The method of claim 60 wherein the patient achieves a reduction in central retinal thickness at 24 weeks following the initial dose.

63. (**New**) The method of claim 53 wherein exclusion criteria for the patient include (1) active intraocular inflammation; or (2) active ocular or periocular infection.

64. (**New**) The method of claim 63 wherein the patient achieves a gain in visual acuity following treatment.

65. (**New**) The method of claim 64 wherein the patient achieves a gain in Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

66. (**New**) The method of claim 63 wherein the patient achieves a gain in visual acuity at 12 weeks following the initial dose.

67. (**New**) The method of claim 66 wherein the patient achieves a gain in Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

68. (**New**) The method of claim 63 wherein the patient achieves a gain in visual acuity at 24 weeks following the initial dose.

69. (**New**) The method of claim 68 wherein the patient achieves a gain in Best Corrected Visual Acuity (BCVA) according to Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

70. (**New**) The method of claim 63 wherein the patient achieves a reduction in central retinal thickness as measured by optical coherence tomography (OCT) following treatment.

71. (**New**) The method of claim 70 wherein the patient achieves a reduction in central retinal thickness at 12 weeks following the initial dose.

72. (**New**) The method of claim 70 wherein the patient achieves a reduction in central retinal thickness at 24 weeks following the initial dose.

==========

Sequence Listing was accepted.

See attached Validation Report.

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

Reviewer: Anjum, Durreshwar

Timestamp: [year=2020; month=10; day=16; hr=15; min=21; sec=32; ms=593;]

==========

Validated By CRFValidator v 1.0.5

Application No: 17072417 Version No: 1.0

Input Set:

Output Set:

Started: 2020-10-16 14:01:20.948
Finished: 2020-10-16 14:01:21.111
Elapsed: 0 hr(s) 0 min(s) 0 sec(s) 163 ms
Total Warnings: 2
Total Errors: 1
No. of SeqIDs Defined: 2
Actual SeqID Count: 2

Error code		Error Description
E	287	Invalid WIPO ST.2 date format; Use (YYYY-MM-DD) in <141>
W	213	Artificial or Unknown found in <213> in SEQ ID (1)
W	213	Artificial or Unknown found in <213> in SEQ ID (2)

SEQUENCE LISTING

<110> George D. Yancopoulos

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

<130> REGN-008CIPCON6

<140> US 17/072,417
<141> 2020-10-16

<150> 16/055,847
<151> 2018-08-06

<150> 16/397,267
<151> 2019-04-29

<150> 16/159,272
<151> 2018-10-12

<150> 15/471,506
<151> 2017-03-28

<150> 14/972,560
<151> 2015-12-17

<150> 13/940,370
<151> 2013-07-12

<150> PCT/US2012/020855
<151> 2012-01-11

<150> 61/432,245
<151> 2011-01-13

<150> 61/434,836
<151> 2011-01-21

<150> 61/561,957
<151> 2011-11-21

<160> 2

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 1377
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 1
atggtcagct actgggacac cggggtcctg ctgtgcgcgc tgctcagctg tctgcttctc 60
acaggatcta gttccggaag tgataccggt agacctttcg tagagatgta cagtgaaatc 120

```
cccgaaatta tacacatgac tgaaggaagg gagctcgtca ttccctgccg ggttacgtca 180
cctaacatca ctgttacttt aaaaaagttt ccacttgaca ctttgatccc tgatggaaaa 240
cgcataatct gggacagtag aaagggcttc atcatatcaa atgcaacgta caaagaaata 300
gggcttctga cctgtgaagc aacagtcaat gggcatttgt ataagacaaa ctatctcaca 360
catcgacaaa ccaatacaat catagatgtg gttctgagtc cgtctcatgg aattgaacta 420
tctgttggag aaaagcttgt cttaaattgt acagcaagaa ctgaactaaa tgtggggatt 480
gacttcaact gggaataccc ttcttcgaag catcagcata agaaacttgt aaaccgagac 540
ctaaaaaccc agtctgggag tgagatgaag aaatttttga gcaccttaac tatagatggt 600
gtaacccgga gtgaccaagg attgtacacc tgtgcagcat ccagtgggct gatgaccaag 660
aagaacagca catttgtcag ggtccatgaa aaggacaaaa ctcacacatg cccaccgtgc 720
ccagcacctg aactcctggg gggaccgtca gtcttcctct tccccccaaa acccaaggac 780
accctcatga tctcccggac ccctgaggtc acatgcgtgg tggtggacgt gagccacgaa 840
gaccctgagg tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca 900
aagccgcggg aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg 960
caccaggact ggctgaatgg caaggagtac aagtgcaagg tctccaacaa agccctccca 1020
gcccccatcg agaaaaccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac 1080
accctgcccc catcccggga tgagctgacc aagaaccagg tcagcctgac ctgcctggtc 1140
aaaggcttct atcccagcga catcgccgtg gagtgggaga gcaatgggca gccggagaac 1200
aactacaaga ccacgcctcc cgtgctggac tccgacggct ccttcttcct ctacagcaag 1260
ctcaccgtgg acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgcat 1320
gaggctctgc acaaccacta cacgcagaag agcctctccc tgtctccggg taaatga    1377
```

<210> 2
<211> 458
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 2

```
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
 1               5                  10                  15
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Asp Thr Gly Arg Pro
            20                  25                  30
Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu
        35                  40                  45
Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr
    50                  55                  60
Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys
65                  70                  75                  80
Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
                85                  90                  95
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
            100                 105                 110
Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile
        115                 120                 125
Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
    130                 135                 140
Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
145                 150                 155                 160
Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
                165                 170                 175
Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
            180                 185                 190
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
        195                 200                 205
```

```
Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
    210                 215                 220
Phe Val Arg Val His Glu Lys Asp Lys Thr His Thr Cys Pro Pro Cys
225                 230                 235                 240
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
                245                 250                 255
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
        275                 280                 285
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
    290                 295                 300
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
305                 310                 315                 320
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
                325                 330                 335
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
            340                 345                 350
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
        355                 360                 365
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
    370                 375                 380
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
385                 390                 395                 400
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
                405                 410                 415
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
            420                 425                 430
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
        435                 440                 445
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455
```

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 17/072,417

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	320
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	700
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	800
TOTAL CLAIMS (37 CFR 1.16(i))	52 minus 20 =	* 32			OR	x 100 =	3200
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*				x 480 =	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	5020

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	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
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	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
17/072,417	10/16/2020		5020	REGN-008CIPCON6	52	3

CONFIRMATION NO. 7325

96387
Regeneron - Bozicevic, Field & Francis
201 REDWOOD SHORES PARKWAY
SUITE 200
REDWOOD CITY, CA 94065

FILING RECEIPT

OC000000120862570

Date Mailed: 10/23/2020

Receipt is acknowledged of this non-provisional utility 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 FIRST INVENTOR, 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 corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction 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 provided that the request is grantable.

Inventor(s)

George D. YANCOPOULOS, Yorktown Heights, NY;

Applicant(s)

REGENERON PHARMACEUTICALS, INC., Tarrytown, NY

Assignment For Published Patent Application

REGENERON PHARMACEUTICALS, INC., Tarrytown, NY

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 16/055,847 08/06/2018
and is a CON of 16/397,267 04/29/2019
which is a CON of 16/159,282 10/12/2018 PAT 10828345
which is a CON of 15/471,506 03/28/2017 PAT 10130681
which is a CON of 14/972,560 12/17/2015 PAT 9669069
which is a CON of 13/940,370 07/12/2013 PAT 9254338
which 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 Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 10/22/2020

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 17/072,417**

Projected Publication Date: 01/28/2021

Non-Publication Request: No

Early Publication Request: No
Title

USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

Preliminary Class

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.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

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.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign

patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

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
17/072,417	10/16/2020	George D. YANCOPOULOS	REGN-008CIPCON6

CONFIRMATION NO. 7325

96387
Regeneron - Bozicevic, Field & Francis
201 REDWOOD SHORES PARKWAY
SUITE 200
REDWOOD CITY, CA 94065

PUBLICATION NOTICE

OC00000122975789

Title:USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

Publication No.US-2021-0023173-A1
Publication Date:01/28/2021

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 Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, 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 https://portal.uspto.gov/pair/PublicPair. 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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	17/072,417
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	1	of	1	Attorney Docket Number	REGN-008CIPCON6

U.S. PATENT DOCUMENTS					
Examiner Initial*	Cite No.	Patent Number Number-Kind Code (*if known*)	Issue Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1				

U.S. PATENT APPLICATION PUBLICATIONS					
Examiner Initial*	Cite No.	Publication Number **Number-Kind Code** (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1				

FOREIGN PATENT DOCUMENTS						
Examiner Initial*	Cite No.	Foreign Document Number Country Code-Number-Kind Code (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	1					

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	1	HEIER, J., "Intravitreal VEGF Trap for AMD: An Update, The CLEAR-IT 2 Extension Study" Presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Retina Today (2009) pp. 44-45		

Examiner Signature		Date Considered	

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

Electronic Acknowledgement Receipt

EFS ID:	43016291
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	17-JUN-2021
Filing Date:	16-OCT-2020
Time Stamp:	13:49:46
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-008CIPCON6_2020-06-17_SuppIDS_Trans.pdf	52780 ee8bcb7596678471c40fff4a93452f85bc8fcb56	no	3

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	REGN-008CIPCON6_2021-06-17_SuppIDS_SB08A.pdf	22856 dda3a70228b82a819f1b11e5d1a5345dfc5f5c65	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Non Patent Literature	Heier_10-2009.pdf	73295 7c554f5fad491f0c5d0a6cf8043ea82a80933396	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	148931	

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 6/17/2021

INFORMATION DISCLOSURE STATEMENT	Attorney Docket No.	REGN-008CIPCON6
	Confirmation No.	7325
	First Named Inventor	George D. Yancopoulos
	Application Number	17/072,417
	Filing Date	October 16, 2020
	Group Art Unit	To Be Assigned
	Examiner Name	To Be Assigned
Address to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

Applicant submits 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 publications discussed herein are provided to comply with the duty to disclose in accordance with 37 C.F.R. § 1.56. However, nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed

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 documents 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-008CIPCON6.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: June 17, 2021 By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
Reg. No. 28,807

BOZICEVIC, FIELD & FRANCIS LLP
201 Redwood Shores Parkway, Suite 200
Redwood City, CA 94065
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

U.S. PATENT DOCUMENTS					
		DOCUMENT NUMBER	DATE	NAME	REFERENCE PROVIDED*
	1	6,171,586	1/9/2001	Lam *et al.*	not required per 69 Fed. Reg. 56481
	2	7,303,747	12/4/2007	Wiegand *et al.*	not required per 69 Fed. Reg. 56481
	3	7,374,757	5/20/2008	Papadopoulos *et al.*	not required per 69 Fed. Reg. 56481
	4	7,374,758	5/20/2008	Papadopoulos *et al.*	not required per 69 Fed. Reg. 56481
	5	7,378,095	5/27/2008	Cao *et al.*	not required per 69 Fed. Reg. 56481
	6	7,521,049	4/21/2009	Wiegand *et al.*	not required per 69 Fed. Reg. 56481
	7	7,531,173	5/12/2009	Wiegand *et al.*	not required per 69 Fed. Reg. 56481
	8	10,828,345	11/10/2020	Yancopoulos	not required per 69 Fed. Reg. 56481
	9	2003/0113316	6/19/2003	Kaisheva *et al.*	not required per 69 Fed. Reg. 56481
	10	2003/0138417	7/24/2003	Kaisheva *et al.*	not required per 69 Fed. Reg. 56481
	11	2004/0197324	10/7/2004	Liu *et al.*	not required per 69 Fed. Reg. 56481
	12	2006/0217311	9/28/2006	Dix *et al.*	not required per 69 Fed. Reg. 56481
	13	2016/0130337	5/12/2016	Gekkieva *et al.*	not required per 69 Fed. Reg. 56481

FOREIGN PATENT DOCUMENTS						
		DOCUMENT NUMBER	DATE	COUNTRY	TRANSLATION	REFERENCE PROVIDED*
	14	2663325	11/20/2013	EP	n/a	Herewith
	15	97/04801	2/13/1997	WO	n/a	Herewith

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	16	7,374,758 – Patent Term Extension Application submitted December 22, 2011	Herewith
	17	ADIS R&D Profile "Aflibercept: AVE 0005, AVE 005, AVE0005, VEGF Trap - Regeneron, VEGF Trap (R1R2), VEGF Trap-Eye." Drugs R D, 9(4):261-269 (2008)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	18	Andersen & Krummen, "Recombinant protein expression for therapeutic applications" Current Opinion in Biotechnology 13:117-123 (2002)	Herewith
	19	Anderson *et al.*, "Delivery of Anti-Angiogenic Molecular Therapies for Retinal Disease" Drug Discovery Today 15: 272 (2010)	Herewith
	20	Article in Retinal Physician, "Subspecialty News", available online at http://www.retinalphysician.com/printarticle.aspx?articleID=104007 (March 2010)	Herewith
	21	Ass'n for Res. Vision & Ophthalmology, ARVO® News (Summer 2007)	Herewith
	22	Ass'n for Res. Vision & Ophthalmology, ARVO® News (Winter/Spring 2008)	Herewith
	23	AVASTIN® label	Herewith
	24	Avery, R. L., D. J. Pieramici, M. D. Rabena, A. A. Castellarin, M. A. Nasir and M. J. Giust, "Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration" Ophthalmology 113(3): 363-372 e365 (2006)	Herewith
	25	Bashshur *et al.*, "Intravitreal Bevacizumab for the Management of Choroidal Neovascularization in Age-Related Macular Degeneration" Am J. Ophthalmology 142: 1 (2006)	Herewith
	26	Bayer Press Release, "Bayer and Regeneron Dose First Patient in Second Phase 3 Study for VEGF Trap-Eye in Wet Age-Related Macular Degeneration." May 8, 2008	Herewith
	27	Bayer Press Release, "VEGF Trap-Eye Shows Positive Results in Phase II Study in Patients with Diabetic Macular Edema" February 18, 2010	Herewith
	28	Bayer Press Release, "Bayer HealthCare and Regeneron Announce Encouraging 32-Week Follow Up Results From A Phase 2 Study of VEGF Trap-Eye in Age-Related Macular Degeneration" April 28, 2008	Herewith
	29	Bayer Press Release "Bayer HealthCare and Regeneron Announce VEGF Trap-Eye Achieved Durable Improvement in Vision Over 52 Weeks in a Phase 2 Study in Patients with Age-Related Macular Degeneration" August 19, 2008	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	30	BMJ Publishing Group Ltd., "Review: Ranibizumab (Lucentis) In Neovascular Age-Related Macular Degeneration: Evidence From Clinical Trials" British J. Ophthalmology (December 2020), https://bjo.bmj.com/content/94/1/2.altmetrics	Herewith
	31	Bontempo, "Preformulation Development of Parenteral Biopharmaceuticals" Drugs and the Pharmaceutical Sciences 85:91-108 (1997)	Herewith
	32	Bressler, N. M. and G. Treatment of Age-Related Macular Degeneration with Photodynamic Therapy Study, "Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: two-year results of 2 randomized clinical trials-tap report 2." Arch Ophthalmol 119(2): 198-207 (2001)	Herewith
	33	Brown & Regillo, "Anti-VEGF Agents in the Treatment of Neovascular Age-Related Macular Degeneration: Applying Clinical Trial Results to the Treatment of Everyday Patients" Am J. Ophthalmology 144: 627 (2007)	Herewith
	34	Chi *et al.*, "Physical Stability of Proteins in Aqueous Solution: Mechanism and Driving Forces in Nonnative Protein Aggregation" Pharmaceutical Research Vol. 20, No. 9, 1325-1336 (September 2003)	Herewith
	35	Ciulla & Rosenfeld, "Antivascular Endothelial Growth Factor Therapy For Neovascular Age-Related Macular Degeneration" Current Opinion Ophthalmology 20: 158 (2009)	Herewith
	36	Clinicaltrials.gov. I-SPY 2 TRIAL: Neoadjuvant and Personalized Adaptive Novel Agents to Treat Breast Cancer, Accessed 2010; http://clinical trials.gov/ct2/show/NCT01042379?term-NCT01042379&rank=1	Herewith
	37	CMS, Local Coverage Determination (LCD) for Ranibizumab (Lucentis) (L29266, First Coast Service Options, Inc June 14, 2011)	Herewith
	38	Controls in SCI experiments, RegenBase. Retrieved January 6, 2021, from http://regenbase.org/control-groups.html	Herewith
	39	Department of Health and Human Services, Office of Inspector General, "Questionable Billing for Medicare Ophthalmology Services" September 2015 OEI-04-12-00280	Herewith
	40	Drug Vehicle (Code C927), National Cancer Institute (NCI). Retrieved January 6, 2021, from https://ncithesaurus.nci.nih.gov/ncitbrowser/ConceptReport.jsp?dictionary =NCI_Thesaurus&code=C927&ns=ncit	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	41	EP 2 663 325 File History	Herewith
	42	Eylea® Prescribing Information, Revised 05/2019	Herewith
	43	Ferrara, N. & Kerbel, R., "Angiogenesis as a Therapeutic Target" Nature 438: 967 (2005)	Herewith
	44	Fraser *et al.*, "Single Injections of Vascular Endothelial Growth Factor Trap Block Ovulation in the Macaque and Produce a Prolonged, Dose-Related Suppression of Ovarian Function." J. Clin. Endocrinol & Metab. 90(2): 1114-1122 (February 2005)	Herewith
	45	Genentech, "FDA Approves Lucentis for the Treatment of Wet Age-Related Macular Degeneration," News Release dated June 30, 2006 (June 30, 2006)	Herewith
	46	Gupta, O. P., G. Shienbaum, A. H. Patel, C. Fecarotta, R. S. Kaiser and C. D. Regillo, "A treat and extend regimen using ranibizumab for neovascular age-related macular degeneration clinical and economic impact" Ophthalmology 117(11): 2134-2140 (2010)	Herewith
	47	Heier, "Intravitreal VEGF Trap for AMD: An Update" Retina Today 44 (October 2009)	Herewith
	48	Heier, J. S., P. A. Campochiaro, L. Yau, Z. Li, N. Saroj, R. G. Rubio and P. Lai "Ranibizumab for macular edema due to retinal vein occlusions: long-term follow-up in the HORIZON trial" Ophthalmology 119(4): 802-809 (2012)	Herewith
	49	HERCEPTIN® label	Herewith
	50	Holz *et al.*, "VEGF Trap-Eye for Macular Oedema Secondary to Central Retinal Vein Occlusion: 6-Month Results of the Phase III GALILEO Study" British J. Ophthalmology 97: 278 (2013)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	51	Ip, M. S., I. U. Scott, P. C. VanVeldhuisen, N. L. Oden, B. A. Blodi, M. Fisher, L. J. Singerman, M. Tolentino, C. K. Chan, V. H. Gonzalez and S. S. R. Group "A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with observation to treat vision loss associated with macular edema secondary to central retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 5" Arch Ophthalmol 127(9): 1101-1114 (2009)	Herewith
	52	Janeway *et al.*, "The structure of a typical antibody molecule" Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science (2001)	Herewith
	53	Keane *et al.*, "Effect of Ranibizumab Retreatment Frequency on Neurosensory Retinal Volume in Neovascular AMD" Retina 29: 592 (2009)	Herewith
	54	Kim *et al.*, "Potent VEGF Blockade Causes Regression of Coopted Vessels in a Model of Neuroblastoma" Proc. Nat'l Acad. Sci. 99: 11399 (2002)	Herewith
	55	LUCENTIS Approval (2006)	Herewith
	56	LUCENTIS® Label (14 pages)	Herewith
	57	LUCENTIS® Prescribing Information (2006)	Herewith
	58	Macular Photocoagulation Study, G., "Laser photocoagulation of subfoveal neovascular lesions in age-related macular degeneration. Results of a randomized clinical trial. Macular Photocoagulation Study Group" Arch Ophthalmol 109(9): 1220-1231 (1991)	Herewith
	59	Massin, "Anti-VEGF Therapy for Diabetic Macular Edema: An Update" Retina Today 54 (Sept./Oct. 2008)	Herewith
	60	Michels, S., P. J. Rosenfeld, C. A. Puliafito, E. N. Marcus and A. S. Venkatraman, "Systemic bevacizumab (Avastin) therapy for neovascular age-related macular degeneration twelve-week results of an uncontrolled open-label clinical study" Ophthalmology 112(6): 1035-1047 (2005)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	61	Mitchell *et al.*, "Ranibizumab (Lucentis) in Neovascular Age-Related Macular Degeneration: Evidence from Clinical Trials" Brit. J. Ophthalmology 94: 2 (2009)	Herewith
	62	Ni & Hui, "Emerging Pharmacologic Therapies for Wet Age-Related Macular Degeneration" Ophthalmologica 223: 401 (2009)	Herewith
	63	Parkins & Lashmar, "The formulation of biopharmaceutical products" Pharmaceutical Science & Technology Today Vol. 3, No. 4: 129-137 (April 4, 2000)	Herewith
	64	Phosphate buffer. Cold Spring Harbor Protocols 2006: pdb.rec8543 (2006)	Herewith
	65	Randolph & Jones, "Surfactant-Protein Interactions" Rational Design of Stable Protein Formulations pp. 159-175, Springer, Boston, MA (2002)	Herewith
	66	RAPTIVA® label	Herewith
	67	Regeneron Pharmaceuticals Inc. Regeneron Receives $20 Million Milestone Payment for Initiation of Phase 3 Study of VEGF Trap-Eye in Wet AMD. Media Release: 14 Aug 2007. Available from URL: http://www.regeneron.com	Herewith
	68	Regeneron Pharmaceuticals Inc. Regeneron Reports Fourth Quarter and Full Year 2004 Financial and Operating Results. Media Release: 22 Feb 2005. Available from URL: http://www.regeneron.com	Herewith
	69	Regeneron Pharmaceuticals Inc. Regeneron Reports Fourth Quarter and Full Year 2005 Financial and Operating Results. Media Release: 24 Feb 2006. Available from URL: http://regeneron.com	Herewith
	70	Regeneron Pharmaceuticals Inc. Regeneron Reports Positive Phase Data for the VEGF Trap in Age-Related Macular Degeneration; Preliminary Results Show Improvements in Vision and Reginal Swelling; VEGF Trap Was Well Tolerated at All Dose Levels. Media Release: 1 May 2006. Available from URL: http://www.regeneron.com	Herewith
	71	Regeneron SEC Form 10-Q (September 30, 2009)	Herewith
	72	Reichert, "Antibody-Based Therapeutics To Watch In 2011" MABS 3: 76 (2011)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	73	REMICADE® label	Herewith
	74	Retina Coding Q & A, Retinal Physician, 16: 18, 54 (July/August 2019)	Herewith
	75	Rogers *et al.*, "The prevalence of retinal vein occlusion: pooled data from population studies from the United States, Europe, Asia, and Australia" Ophthalmology 117(2): 313-319 e311 (2010)	Herewith
	76	Rosenfeld, P. J., A. A. Moshfeghi and C. A. Puliafito, "Optical coherence tomography findings after an intravitreal injection of bevacizumab (avastin) for neovascular age-related macular degeneration" Ophthalmic Surg Lasers Imaging 36(4): 331-335 (2005)	Herewith
	77	Rudge *et al.*, "VEGF Trap as a Novel Antiangiogenic Treatment Currently in Clinical Trials for Cancer and Eye Diseases, and VelociGene®-based Discovery of the Next Generation of Angiogenesis Targets," Cold Spring Harbor Symposia on Quantitative Biology 70: 411-418 (2005)	Herewith
	78	Schmidt-Erfurth "Current Concepts in the Management of Diabetic Macular Edema" Proceedings 7:52 (2010)	Herewith
	79	Scott *et al.*, "A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with standard care to treat vision loss associated with macular Edema secondary to branch retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 6" Arch Ophthalmol 127(9): 1115-1128 (2009)	Herewith
	80	SIMULECT® label	Herewith
	81	Spaide *et al.*, "Prospective Study of Intravitreal Ranibizumab as a Treatment for Decreased Visual Acuity Secondary to Central Retinal Vein Occlusion" Am J. Ophthalmology 147: 298 (2009)	Herewith
	82	Spielberg, L. & Leys, A., "Intravitreal Bevacizumab for Myopic Choroidal Neovascularization: Short-Term and 1-Year Results" Bulletin Societe Belge D'Ophtalmologie 312: 17 (2009)	Herewith
	83	Steinbrook, "The Price of Sight — Ranibizumab, Bevacizumab, and the Treatment of Macular Degeneration" N. Eng. J. Med. 355:1409 (2006)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	84	The Branch Vein Occlusion Study, G., "Argon laser photocoagulation for macular edema in branch vein occlusion" Am J Ophthalmol 98(3): 271-282 (1984)	Herewith
	85	The Central Vein Occlusion Study, G., "Evaluation of grid pattern photocoagulation for macular edema in central vein occlusion. The Central Vein Occlusion Study Group M report" Ophthalmology 102(10): 1425-1433 (1995)	Herewith
	86	U.S. DEP'T HEALTH & HUMAN SERVS., NAT'L INST. HEALTH, NAT'L EYE INST., "Age-Related Macular Degeneration: What You Should Know" (Sept. 2015) https://www.nei.nih.gov/sites/default/files/healthpdfs/WYSK_AMD_English_Sept2015_PRINT.pdf	Herewith
	87	U.S. DEP'T HEALTH & HUMAN SERVS., NAT'L INST. HEALTH, NAT'L EYE INST., "Diabetic Retinopathy: What You Should Know" (Sept. 2015), https://www.nei.nih.gov/sites/default/files/2019-06/Diabetic-Retinopathy-What-You-Should-Know-508.pdf	Herewith
	88	U.S. Department of Health and Human Services Food and Drug Administration, "Guidance for industry Q1A(R2) stability testing of new drug substances and products" Rockville, MD (November 2003)	Herewith
	89	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) (VIEW1), NCT00509795, ClinicalTrials.gov (Apr. 28, 2009), https://clinicaltrials.gov/ct2/show/NCT00509795 ("NCT-795")	Herewith
	90	Wall Street Journal, "Genentech's Big Drug for Eyes Faces a Rival" (2007)	Herewith
	91	Wulff *et al.,* "Prevention of Thecal Angiogenesis, Antral Follicular Growth, and Ovulation in the Primate by Treatment with Vascular Endothelial Growth Factor Trap R1R2" Endocrinology 143(7): 2797-2807 (July 2002)	Herewith
	92	XOLAIR® label	Herewith
	93	Zarbin & Rosenfeld, "Pathway-Based Therapies for Age-Related Macular Degeneration: An Integrated Survey of Emerging Treatment Alternatives" Retina 30: 1350 (2010)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 39/00, 39/395, C07K 1/00

A1

(11) International Publication Number: **WO 97/04801**

(43) International Publication Date: 13 February 1997 (13.02.97)

(21) International Application Number: PCT/US96/12251

(22) International Filing Date: 23 July 1996 (23.07.96)

(30) Priority Data:

08/508,014	27 July 1995 (27.07.95)	US
08/615,369	14 March 1996 (14.03.96)	US

(71) Applicant: GENENTECH, INC. [US/US]; 460 Point San Bruno Boulevard, South San Francisco, CA 94080-4990 (US).

(72) Inventors: ANDYA, James; Apartment D, 227 Richmond Drive, Millbrae, CA 94030 (US). CLELAND, Jeffrey, L.; 844 Cordilleras Avenue, San Carlos, CA 94070 (US). HSU, Chung, C.; 13120 Delson Court, Los Altos Hills, CA 94022 (US). LAM, Xanthe, M.; 280 Denslowe Drive, San Francisco, CA 94132 (US). OVERCASHIER, David, E.; 130 Vallejo Street, El Granada, CA 94018 (US). SHIRE, Steven, J.; 2417 Lincoln Avenue, Belmont, CA 94002 (US). YANG, Janet, Yu-Feng; 1860 Dale Avenue, San Mateo, CA 94401 (US). WU, Sylvia, Sau-Yan; 1438 Filbert Street #203, San Francisco, CA 94109 (US).

(74) Agents: LEE, Wendy, M. et al.; Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080-4990 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, 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 the receipt of amendments.

(54) Title: STABILE ISOTONIC LYOPHILIZED PROTEIN FORMULATION

(57) Abstract

A stable lyophilized protein formulation is described which can be reconstituted with a suitable diluent to generate a high protein concentration reconstituted formulation which is suitable for subcutaneous administration. For example, anti-IgE and anti-HER2 antibody formulations have been prepared by lyophilizing these antibodies in the presence of a lyoprotectant. The lyophilized mixture thus formed is reconstituted to a high protein concentration without apparent loss of stability of the protein.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

Stabile isotonic lyophilized protein formulation

Background of the Invention

Field of the Invention

This invention is directed to a lyophilized protein formulation. In particular, it relates to a stable lyophilized protein formulation which can be reconstituted with a diluent to generate a stable reconstituted formulation suitable for subcutaneous administration.

Description of Related Disclosures

In the past ten years, advances in biotechnology have made it possible to produce a variety of proteins for pharmaceutical applications using recombinant DNA techniques. Because proteins are larger and more complex than traditional organic and inorganic drugs (*i.e.* possessing multiple functional groups in addition to complex three-dimensional structures), the formulation of such proteins poses special problems. For a protein to remain biologically active, a formulation must preserve intact the conformational integrity of at least a core sequence of the protein's amino acids while at the same time protecting the protein's multiple functional groups from degradation. Degradation pathways for proteins can involve chemical instability (*i.e.* any process which involves modification of the protein by bond formation or cleavage resulting in a new chemical entity) or physical instability (*i.e.* changes in the higher order structure of the protein). Chemical instability can result from deamidation, racemization, hydrolysis, oxidation, beta elimination or disulfide exchange. Physical instability can result from denaturation, aggregation, precipitation or adsorption, for example. The three most common protein degradation pathways are protein aggregation, deamidation and oxidation. Cleland *et al. Critical Reviews in Therapeutic Drug Carrier Systems* 10(4): 307-377 (1993).

Freeze-drying is a commonly employed technique for preserving proteins which serves to remove water from the protein preparation of interest. Freeze-drying, or lyophilization, is a process by which the material to be dried is first frozen and then the ice or frozen solvent is removed by sublimation in a vacuum environment. An excipient may be included in pre-lyophilized formulations to enhance stability during the freeze-drying process and/or to improve stability of the lyophilized product upon storage. Pikal, M. *Biopharm.* 3(9)26-30 (1990) and Arakawa *et al. Pharm. Res.* 8(3):285-291 (1991).

It is an object of the present invention to provide a lyophilized protein formulation which is stable upon storage and delivery. It is a further object to provide a stable reconstituted protein formulation which is suitable for subcutaneous administration. In certain embodiments, it is an object to provide a multi-use formulation which is stable for at least the time over which it will be administered to a patient.

Summary of the Invention

This invention is based on the discovery that a stable lyophilized protein formulation can be prepared using a lyoprotectant (preferably a sugar such as sucrose or trehalose), which lyophilized formulation can be reconstituted to generate a stable reconstituted formulation having a protein concentration which is significantly higher (*e.g.* from about 2-40 times higher, preferably 3-10 times higher and most preferably 3-6 times higher) than the protein concentration in the pre-lyophilized formulation. In particular, while the protein concentration in the pre-lyophilized formulation may be 5 mg/mL or less, the protein concentration in the reconstituted formulation is generally 50 mg/mL or more. Such high protein concentrations in the reconstituted formulation are considered to be particularly useful where the formulation is intended for subcutaneous administration.

Despite the very high protein concentration in the reconstituted formulation, it has been found that the reconstituted formulation is stable (*i.e.* fails to display significant or unacceptable levels of chemical or physical instability of the protein) at 2-8°C for at least about 30 days. In certain embodiments, the reconstituted formulation is isotonic. In spite of the use of lower concentrations of the lyoprotectant to achieve such isotonic formulations upon reconstitution, it was discovered herein that the protein in the lyophilized formulation essentially retains its physical and chemical stability and integrity upon lyophilization and storage.

When reconstituted with a diluent comprising a preservative (such as bacteriostatic water for injection, BWFI), the reconstituted formulation may be used as a multi-use formulation. Such a formulation is useful, for example, where the patient requires frequent subcutaneous administrations of the protein to treat a chronic medical condition. The advantage of a multi-use formulation is that it facilitates ease of use for the patient, reduces waste by allowing complete use of vial contents, and results in a significant cost savings for the manufacturer since several doses are packaged in a single vial (lower filling and shipping costs).

Based on the observations described herein, in one aspect the invention provides a stable isotonic reconstituted formulation comprising a protein in an amount of at least about 50 mg/mL and a diluent, which reconstituted formulation has been prepared from a lyophilized mixture of a protein and a lyoprotectant, wherein the protein concentration in the reconstituted formulation is about 2-40 times greater than the protein concentration in the mixture before lyophilization.

In another embodiment, the invention provides a stable reconstituted formulation comprising an antibody in an amount of at least about 50 mg/mL and a diluent, which reconstituted formulation has been prepared from a lyophilized mixture of an antibody and a lyoprotectant, wherein the antibody concentration in the reconstituted formulation is about 2-40 times greater than the antibody concentration in the mixture before lyophilization.

The ratio of lyoprotectant:protein in the lyophilized formulation of the preceding paragraphs depends, for example, on both the protein and lyoprotectant of choice, as well as the desired protein concentration and isotonicity of the reconstituted formulation. In the case of a full length antibody (as the protein) and trehalose or sucrose (as the lyoprotectant) for generating a high protein concentration isotonic reconstituted formulation, the ratio may, for example, be about 100-1500 mole trehalose or sucrose:1 mole antibody.

Generally, the pre-lyophilized formulation of the protein and lyoprotectant will further include a buffer which provides the formulation at a suitable pH, depending on the protein in the formulation. For this purpose, it has been found to be desirable to use a histidine buffer in that, as demonstrated below, this appears to have lyoprotective properties.

The formulation may further include a surfactant (*e.g.* a polysorbate) in that it has been observed herein that this can reduce aggregation of the reconstituted protein and/or reduce the formation of particulates in the reconstituted formulation. The surfactant can be added to the pre-lyophilized formulation, the lyophilized formulation and/or the reconstituted formulation (but preferably the pre-lyophilized formulation) as desired.

The invention further provides a method for preparing a stable isotonic reconstituted formulation comprising reconstituting a lyophilized mixture of a protein and a lyoprotectant in a diluent such that the protein concentration in the reconstituted formulation is at least 50 mg/mL, wherein the protein concentration in the

reconstituted formulation is about 2-40 times greater than the protein concentration in the mixture before lyophilization.

In yet a further embodiment, the invention provides a method for preparing a formulation comprising the steps of: (a) lyophilizing a mixture of a protein and a lyoprotectant; and (b) reconstituting the lyophilized mixture of step (a) in a diluent such that the reconstituted formulation is isotonic and stable and has a protein concentration of at least about 50 mg/mL. For example, the protein concentration in the reconstituted formulation may be from about 80 mg/mL to about 300 mg/mL. Generally, the protein concentration in the reconstituted formulation is about 2-40 times greater than the protein concentration in the mixture before lyophilization.

An article of manufacture is also provided herein which comprises: (a) a container which holds a lyophilized mixture of a protein and a lyoprotectant; and (b) instructions for reconstituting the lyophilized mixture with a diluent to a protein concentration in the reconstituted formulation of at least about 50 mg/mL. The article of manufacture may further comprise a second container which holds a diluent (*e.g.* bacteriostatic water for injection (BWFI) comprising an aromatic alcohol).

The invention further provides a method for treating a mammal comprising administering a therapeutically effective amount of a reconstituted formulation disclosed herein to a mammal, wherein the mammal has a disorder requiring treatment with the protein in the formulation. For example, the formulation may be administered subcutaneously.

One useful anti-HER2 antibody pre-lyophilized formulation as discovered in the experiments detailed below was found to comprise anti-HER2 in amount from about 5-40 mg/mL (*e.g.* 20-30 mg/mL) and sucrose or trehalose in an amount from about 10-100 mM (*e.g.* 40-80 mM), a buffer (*e.g.* histidine, pH 6 or succinate, pH 5) and a surfactant (*e.g.* a polysorbate). The lyophilized formulation was found to be stable at 40°C for at least 3 months and stable at 30°C for at least 6 months. This anti-HER2 formulation can be reconstituted with a diluent to generate a formulation suitable for intravenous administration comprising anti-HER2 in an amount from about 10-30 mg/mL which is stable at 2-8°C for at least about 30 days. Where higher concentrations of the anti-HER2 antibody are desired (for example where subcutaneous delivery of the antibody is the intended mode of administration to the patient), the lyophilized formulation may be reconstituted to yield a stable reconstituted formulation having a protein concentration of 50 mg/mL or more.

One desirable anti-IgE antibody pre-lyophilized formulation discovered herein has anti-IgE in amount from about 5-40 mg/mL (*e.g.* 20-30 mg/mL) and sucrose or trehalose in an amount from about 60-300 mM (*e.g.* 80-170 mM), a buffer (preferably histidine, pH 6) and a surfactant (such as a polysorbate). The lyophilized anti-IgE formulation is stable at 30°C for at least 1 year. This formulation can be reconstituted to yield a formulation comprising anti-IgE in an amount from about 15-45 mg/mL (*e.g.* 15-25 mg/mL) suitable for intravenous administration which is stable at 2-8°C for at least 1 year. Alternatively, where higher concentrations of anti-IgE in the formulation are desired, the lyophilized formulation can be reconstituted in order to generate a stable formulation having an anti-IgE concentration of ≥50 mg/mL.

Brief Description of the Drawings

Figure 1 shows the effect of reconstitution volume on the stability of lyophilized rhuMAb HER2. The lyophilized formulation was prepared from a pre-lyophilization formulation comprising 25 mg/mL protein, 60

mM trehalose, 5 mM sodium succinate, pH 5.0, and 0.01% Tween 20™. The lyophilized cake was incubated at 40°C and then reconstituted with 4.0 (○) or 20.0 mL (●) of BWFI. The fraction of intact protein in the reconstituted formulation was measured by native size exclusion chromatography and defined as the peak area of the native protein relative to the total peak area including aggregates.

Figure 2 illustrates the effect of trehalose concentration on the stability of lyophilized rhuMAb HER2. The protein was lyophilized at 25 mg/mL in 5 mM sodium succinate, pH 5.0 (circles) or 5 mM histidine, pH 6.0 (squares) and trehalose concentrations ranging from 60 mM (360 molar ratio) to 200 mM (1200 molar ratio). The lyophilized protein was incubated at 40°C for either 30 days (closed symbols) or 91 days (open symbols). The amount of intact protein was measured after reconstitution of the lyophilized protein with 20 mL BWFI.

Figure 3 demonstrates the effect of trehalose concentration on the long term stability of lyophilized rhuMAb HER2 stored at 40°C. The protein was lyophilized at either 25 mg/mL in 5 mM sodium succinate, pH 5.0, 0.01% Tween 20™, and 60 mM trehalose (■) or 5 mM histidine, pH 6.0, 0.01% Tween 20™, and 60 mM trehalose (□) or 21 mg/mL in 10 mM sodium succinate, pH 5.0, 0.2% Tween 20™ and 250 mM trehalose (●). The lyophilized protein was incubated at 40°C and then reconstituted with 20 mL of BWFI. The amount of intact protein was measured after reconstitution.

Figure 4 shows the stability of rhuMAb HER2 lyophilized in 38.4 mM mannitol (7 mg/mL), 20.4 mM sucrose (7 mg/mL), 5 mM histidine, pH 6.0, 0.01% Tween 20™. The lyophilized protein was incubated at 40°C and then reconstituted with either 4.0 mL (○) or 20 mL (●) of BWFI. The amount of intact protein was measured after reconstitution.

Figure 5 demonstrates stability of reconstituted rhuMAb HER2 lyophilized in 5 mM sodium succinate, pH 5.0, 60 mM trehalose, 0.01% Tween 20™. Samples were reconstituted with either 4.0 mL (squares) or 20.0 mL (circles) of BWFI (20 mL:0.9% benzyl alcohol; 4 mL:1.1% benzyl alcohol) and then stored at 5°C (solid symbols) or 25°C (open symbols). The % native protein was defined as the peak area of the native (not degraded) protein relative to the total peak area as measured by cation exchange chromatography.

Figure 6 shows stability of reconstituted rhuMAb HER2 lyophilized in 5 mM histidine, pH 6.0, 60 mM trehalose, 0.01% Tween 20. Samples were reconstituted with either 4.0 mL (squares) or 20.0 mL (circles) of BWFI (20 mL:0.9% benzyl alcohol; 4 mL:1.1% benzyl alcohol) and then stored at 5°C (solid symbols) or 25 °C (open symbols). The % native protein was defined as the peak area of the native (not degraded) protein relative to the total peak area as measured by cation exchange chromatography.

Figure 7 reveals stability of reconstituted rhuMAb HER2 lyophilized in 5 mM histidine, pH 6.0, 38.4 mM mannitol, 20.4 mM sucrose, 0.01% Tween 20. Samples were reconstituted with either 4.0 mL (squares) or 20.0 mL (circles) of BWFI (20 mL:0.9% benzyl alcohol; 4 mL:1.1% benzyl alcohol) and then stored at 5°C (solid symbols) or 25 °C (open symbols). The % native protein was defined as the peak area of the native (not degraded) protein relative to the total peak area as measured by cation exchange chromatography.

Figure 8 shows stability of reconstituted rhuMAb HER2 lyophilized in 10 mM sodium succinate, pH 5.0, 250 mM trehalose, 0.2% Tween 20. Samples were reconstituted with 20.0 mL of BWFI (0.9% benzyl alcohol) and then stored at 5°C (●) or 25 °C (○). The % native protein was defined as the peak area of the native (not degraded) protein relative to the total peak area as measured by cation exchange chromatography.

Figure 9 shows aggregation of rhuMAb E25 formulated into buffers ranging from pH 5 to pH 7 at 10 mM buffer concentration and 5 mg/mL antibody concentration. Samples were lyophilized and assayed at time zero and after 4 weeks, 8 weeks, and 52 weeks of storage at 2-8° C. The buffers were: potassium phosphate pH 7.0 (○); sodium phosphate pH 7.0 (□); histidine pH 7.0 (◇); sodium succinate pH 6.5 (●); sodium succinate pH 6.0 (■); sodium succinate pH 5.5 (◆); and sodium succinate pH 5.0 (▲).

Figure 10 depicts aggregation of rhuMAb E25 lyophilized in 5 mM histidine buffer at both pH 6 and pH 7 and assayed following storage as follows. The buffer was at: pH 6.0 stored at 2-8° C (○); pH 6 stored at 25° C (□); pH 6 stored at 40° C (◇); pH 7 stored at 2-8° C (●); pH 7 stored at 25° C (■); and pH 7 stored at 40° C (◆).

Figure 11 illustrates aggregation of 5 mg/mL rhuMAb E25 formulated into 10 mM sodium succinate at pH 5.0 with lyoprotectant added at a concentration of 275 mM (isotonic). The lyoprotectants were: control, no lyoprotectant (○); mannitol (□); lactose (◇); maltose (●); trehalose (■); and sucrose (◆). Samples were lyophilized and assayed at time zero and after 4 weeks, 8 weeks, and 52 weeks of storage at 2-8° C.

Figure 12 shows aggregation of 5 mg/mL rhuMAb E25 formulated into 10 mM sodium succinate at pH 5.0 with lyoprotectant added at a concentration of 275 mM (isotonic). The lyoprotectants were: control, no lyoprotectant (○); mannitol (□); lactose (◇); maltose (●); trehalose (■); and sucrose (◆). Samples were lyophilized and assayed at time zero and after 4 weeks, 8 weeks, and 52 weeks of storage at 40° C.

Figure 13 depicts hydrophobic interaction chromatography of 20 mg/mL rhuMAb E25 lyophilized in histidine buffer at pH 6 with an isotonic concentration (*i.e.* 275 mM) of lactose stored for 24 weeks at 2-8, 25 or 40° C and reconstituted to 20 mg/mL.

Figure 14 shows hydrophobic interaction chromatography of 20 mg/mL rhuMAb E25 lyophilized in histidine buffer at pH 6 stored for 24 weeks at 2-8, 25 or 40° C and reconstituted to 20 mg/mL.

Figure 15 illustrates hydrophobic interaction chromatography of 20 mg/mL rhuMAb E25 lyophilized in histidine buffer at pH 6 with an isotonic concentration (*i.e.* 275 mM) of sucrose and stored for 24 weeks at 2-8, 25 or 40° C and reconstituted to 20 mg/mL.

Figure 16 illustrates the effect of sugar concentration on rhuMAb E25 formulated at 20 mg/mL in 5 mM histidine at pH 6.0. Sucrose (●) and trehalose (□) were added to the formulation at molar ratios ranging from 0 to 2010 (isotonic) (see Table 1 below). Samples were lyophilized and assayed after 12 weeks of storage at 50° C.

TABLE 1

Moles of Sugar: E25 antibody	Sugar conc. (mM)
0	0
260	34.4
380	51.6
510	68.8
760	103.1
1020	137.5
1530	206.3

2010	275

Figure 17 reveals aggregation of rhuMAb E25 formulated at 25 mg/mL into 5 mM histidine at pH 6 with 85 mM sucrose (○); 85 mM trehalose (□); 161 mM sucrose (♦) or 161 mM trehalose (▲). Samples were lyophilized and stored at 2-8°C followed by reconstitution with 0.9% benzyl alcohol to 100 mg/mL antibody in 20 mM histidine at pH 6 with isotonic (340 mM) and hypertonic (644 mM) sugar concentration.

Figure 18 shows aggregation of rhuMAb E25 formulated at 25 mg/mL into 5 mM histidine at pH 6 with 85 mM sucrose (○); 85 mM trehalose (□); 161 mM sucrose (♦) or 161 mM trehalose (▲). Samples were lyophilized and stored at 30°C followed by reconstitution with 0.9% benzyl alcohol to 100 mg/mL antibody in 20 mM histidine at pH 6 with isotonic (340 mM) and hypertonic (644 mM) sugar concentration.

Figure 19 illustrates aggregation of rhuMAb E25 formulated at 25 mg/mL into 5 mM histidine at pH 6 with 85 mM sucrose (○); 85 mM trehalose (□); 161 mM sucrose (♦) or 161 mM trehalose (▲). Samples were lyophilized and stored at 50°C followed by reconstitution with 0.9% benzyl alcohol to 100 mg/mL antibody in 20 mM histidine at pH 6 with isotonic (340 mM) and hypertonic (644 mM) sugar concentration.

Detailed Description of the Preferred Embodiments

I. Definitions

By "protein" is meant a sequence of amino acids for which the chain length is sufficient to produce the higher levels of tertiary and/or quaternary structure. This is to distinguish from "peptides" or other small molecular weight drugs that do not have such structure. Typically, the protein herein will have a molecular weight of at least about 15-20 kD, preferably at least about 20 kD.

Examples of proteins encompassed within the definition herein include mammalian proteins, such as, *e.g.*, growth hormone, including human growth hormone and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; α-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or tissue-type plasminogen activator (t-PA); bombazine; thrombin; tumor necrosis factor-α and -β; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-α); serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; an integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-α and TGF-β, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I); insulin-like growth factor binding proteins; CD proteins such as CD3, CD4, CD8, CD19 and CD20; erythropoietin (EPO); thrombopoietin (TPO); osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-α, -β, and -γ; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1 to IL-10; superoxide

dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor (DAF); a viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; immunoadhesins; antibodies; and biologically active fragments or variants of any of the above-listed polypeptides.

The protein which is formulated is preferably essentially pure and desirably essentially homogeneous (*i.e.* free from contaminating proteins etc). "Essentially pure" protein means a composition comprising at least about 90% by weight of the protein, based on total weight of the composition, preferably at least about 95% by weight. "Essentially homogeneous" protein means a composition comprising at least about 99% by weight of protein, based on total weight of the composition.

In certain embodiments, the protein is an antibody. The antibody may bind to any of the above-mentioned molecules, for example. Exemplary molecular targets for antibodies encompassed by the present invention include CD proteins such as CD3, CD4, CD8, CD19, CD20 and CD34; members of the HER receptor family such as the EGF receptor, HER2, HER3 or HER4 receptor; cell adhesion molecules such as LFA-1, Mo1, p150,95, VLA-4, ICAM-1, VCAM and $\alpha v/\beta 3$ integrin including either α or β subunits thereof (*e.g.* anti-CD11a, anti-CD18 or anti-CD11b antibodies); growth factors such as VEGF; IgE; blood group antigens; flk2/flt3 receptor; obesity (OB) receptor; protein C etc.

The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies (including full length antibodies which have an immunoglobulin Fc region), antibody compositions with polyepitopic specificity, bispecific antibodies, diabodies, and single-chain molecules, as well as antibody fragments (*e.g.*, Fab, $F(ab')_2$, and Fv).

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. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. 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.

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; Morrison *et al., Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)).

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize 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 CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally 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). The humanized antibody includes a Primatized™ antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest.

A "stable" formulation is one in which the protein therein essentially retains its physical and chemical stability and integrity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in *Peptide and Protein Drug Delivery*, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, New York, Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10: 29-90 (1993). Stability can be measured at a selected temperature for a selected time period. For rapid screening, the formulation may be kept at 40°C for 2 weeks to 1 month, at which time stability is measured. Where the formulation is to be stored at 2-8°C, generally the formulation should be stable at 30°C or 40°C for at least 1 month and/or stable at 2-8°C for at least 2 years. Where the formulation is to be stored at 30°C, generally the formulation should be stable for at least 2 years at 30°C and/or stable at 40°C for at least 6 months. For example, the extent of aggregation following lyophilization and storage can be used as an indicator of protein stability (see Examples herein). For example, a "stable" formulation may be one wherein less than about 10% and preferably less than about 5% of the protein is present as an aggregate in the formulation. In other embodiments, any increase in aggregate formation following lyophilization and storage of the lyophilized formulation can be determined. For example, a "stable" lyophilized formulation may be one wherein the increase in aggregate in the lyophilized formulation is less than about 5% and preferably less than about 3%, when the lyophilized formulation is stored at 2-8°C for at least one year. In other embodiments, stability of the protein formulation may be measured using a biological activity assay (see, *e.g.*, Example 2 below).

A "reconstituted" formulation is one which has been prepared by dissolving a lyophilized protein formulation in a diluent such that the protein is dispersed in the reconstituted formulation. The reconstituted formulation in suitable for administration (*e.g.* parenteral administration) to a patient to be treated with the protein of interest and, in certain embodiments of the invention, may be one which is suitable for subcutaneous administration.

By "isotonic" is meant that the formulation of interest has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350mOsm. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

A "lyoprotectant" is a molecule which, when combined with a protein of interest, significantly prevents or reduces chemical and/or physical instability of the protein upon lyophilization and subsequent storage. Exemplary lyoprotectants include sugars such as sucrose or trehalose; an amino acid such as monosodium glutamate or histidine; a methylamine such as betaine; a lyotropic salt such as magnesium sulfate; a polyol such as trihydric or higher sugar alcohols, *e.g.* glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; Pluronics; and combinations thereof. The preferred lyoprotectant is a non-reducing sugar, such as trehalose or sucrose.

The lyoprotectant is added to the pre-lyophilized formulation in a "lyoprotecting amount" which means that, following lyophilization of the protein in the presence of the lyoprotecting amount of the lyoprotectant, the protein essentially retains its physical and chemical stability and integrity upon lyophilization and storage.

The "diluent" of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a reconstituted formulation. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.* phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

A "preservative" is a compound which can be added to the diluent to essentially reduce bacterial action in the reconstituted formulation, thus facilitating the production of a multi-use reconstituted formulation, for example. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol. The most preferred preservative herein is benzyl alcohol.

A "bulking agent" is a compound which adds mass to the lyophilized mixture and contributes to the physical structure of the lyophilized cake (*e.g.* facilitates the production of an essentially uniform lyophilized cake which maintains an open pore structure). Exemplary bulking agents include mannitol, glycine, polyethylene glycol and xorbitol.

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

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

A "disorder" is any condition that would benefit from treatment with the protein. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include carcinomas and allergies.

II. Modes for Carrying out the Invention

A. Protein Preparation

The protein to be formulated is prepared using techniques which are well established in the art including synthetic techniques (such as recombinant techniques and peptide synthesis or a combination of these techniques) or may be isolated from an endogenous source of the protein. In certain embodiments of the invention, the protein of choice is an antibody. Techniques for the production of antibodies follow.

(i) Polyclonal antibodies.

Polyclonal antibodies are generally raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N=C=NR$, where R and R^1 are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining 1 mg or 1 µg of the peptide or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) Monoclonal antibodies.

Monoclonal antibodies are 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. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al., Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al., Anal. Biochem.*, 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al., Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

In a further embodiment, antibodies can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al., Nature*, 348:552-554 (1990). Clackson *et al., Nature*, 352:624-628 (1991) and Marks *et al., J. Mol. Biol.*, 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity

(nM range) human antibodies by chain shuffling (Marks *et al.*. *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, *et al.*, *Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

Chimeric or hybrid antibodies also may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate. *(iii)*

Humanized and human antibodies.

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeyen *et al.*, *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims *et al.*, *J. Immunol.*, 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.*, 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta *et al.*, *J. Immnol.*, 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized

antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Alternatively, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al.*, *Nature*, 362:255-258 (1993); Bruggermann *et al.*, *Year in Immuno.*, 7:33 (1993). Human antibodies can also be derived from phage-display libraries (Hoogenboom *et al.*, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991)).

(iv) Bispecific antibodies

Bispecific antibodies (BsAbs) are antibodies that have binding specificities for at least two different epitopes. Such antibodies can be derived from full length antibodies or antibody fragments (*e.g.* $F(ab')_2$ bispecific antibodies).

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.*, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in

the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690 published March 3, 1994. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. The following techniques can also be used for the production of bivalent antibody fragments which are not necessarily bispecific. For example, Fab' fragments recovered from *E. coli* can be chemically coupled *in vitro* to form bivalent antibodies. See, Shalaby *et al.*, *J. Exp. Med.*, 175:217-225 (1992).

Various techniques for making and isolating bivalent antibody fragments directly from recombinant cell culture have also been described. For example, bivalent heterodimers have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific/bivalent antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific/bivalent antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, *J. Immunol.*, 152:5368 (1994).

B. Preparation of the Lyophilized Formulation

After preparation of the protein of interest as described above, a "pre-lyophilized formulation" is produced. The amount of protein present in the pre-lyophilized formulation is determined taking into account the desired dose volumes, mode(s) of administration etc. Where the protein of choice is an intact antibody (such as an anti-IgE or anti-HER2 antibody), from about 2 mg/mL to about 50 mg/mL, preferably from about 5 mg/mL to about 40 mg/mL and most preferably from about 20-30 mg/mL is an exemplary starting protein concentration.

The protein is generally present in solution. For example, the protein may be present in a pH-buffered solution at a pH from about 4-8, and preferably from about 5-7. Exemplary buffers include histidine, phosphate, Tris, citrate, succinate and other organic acids. The buffer concentration can be from about 1 mM to about 20 mM, or from about 3 mM to about 15 mM, depending, for example, on the buffer and the desired isotonicity of the formulation (*e.g.* of the reconstituted formulation). The preferred buffer is histidine in that, as demonstrated below, this can have lyoprotective properties. Succinate was shown to be another useful buffer.

The lyoprotectant is added to the pre-lyophilized formulation. In preferred embodiments, the lyoprotectant is a non-reducing sugar such as sucrose or trehalose. The amount of lyoprotectant in the pre-lyophilized formulation is generally such that, upon reconstitution, the resulting formulation will be isotonic. However, hypertonic reconstituted formulations may also be suitable. In addition, the amount of lyoprotectant must not be too low such that an unacceptable amount of degradation/aggregation of the protein occurs upon lyophilization. Where the lyoprotectant is a sugar (such as sucrose or trehalose) and the protein is an antibody, exemplary lyoprotectant concentrations in the pre-lyophilized formulation are from about 10 mM to about 400 mM, and preferably from about 30 mM to about 300 mM, and most preferably from about 50 mM to about 100 mM.

The ratio of protein to lyoprotectant is selected for each protein and lyoprotectant combination. In the case of an antibody as the protein of choice and a sugar (*e.g.,* sucrose or trehalose) as the lyoprotectant for generating an isotonic reconstituted formulation with a high protein concentration, the molar ratio of lyoprotectant to antibody may be from about 100 to about 1500 moles lyoprotectant to 1 mole antibody, and preferably from about 200 to about 1000 moles of lyoprotectant to 1 mole antibody, for example from about 200 to about 600 moles of lyoprotectant to 1 mole antibody.

In preferred embodiments of the invention, it has been found to be desirable to add a surfactant to the pre-lyophilized formulation. Alternatively, or in addition, the surfactant may be added to the lyophilized formulation and/or the reconstituted formulation. Exemplary surfactants include nonionic surfactants such as polysorbates (*e.g.* polysorbates 20 or 80); poloxamers (*e.g.* poloxamer 188); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (*e.g.* lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUAT™ series (Mona Industries, Inc., Paterson, New Jersey), polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (*e.g.* Pluronics, PF68 etc). The amount of surfactant added is such that it reduces aggregation of the reconstituted protein and minimizes the formation of particulates after reconstitution. For example, the surfactant may be present in the pre-lyophilized formulation in an amount from about 0.001-0.5%, and preferably from about 0.005-0.05%.

In certain embodiments of the invention, a mixture of the lyoprotectant (such as sucrose or trehalose) and a bulking agent (*e.g.* mannitol or glycine) is used in the preparation of the pre-lyophilization formulation. The bulking agent may allow for the production of a uniform lyophilized cake without excessive pockets therein etc.

Other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980) may be included in the pre-lyophilized formulation (and/or the lyophilized formulation and/or the reconstituted formulation) provided that they do not adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include: additional buffering agents; preservatives; co-solvents; antioxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (*e.g.* Zn-protein complexes); biodegradable polymers such as polyesters; and/or salt-forming counterions such as sodium.

The formulation herein may also contain more than one protein as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect the other protein. For example, it may be desirable to provide two or more antibodies which bind to the HER2 receptor or IgE in a single formulation. Furthermore, anti-HER2 and anti-VEGF antibodies may be combined in the one formulation. Such proteins are suitably present in combination in amounts that are effective for the purpose intended.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to, or following, lyophilization and reconstitution. Alternatively, sterility of the entire mixture may be accomplished by autoclaving the ingredients, except for protein, at about 120° C for about 30 minutes, for example.

After the protein, lyoprotectant and other optional components are mixed together, the formulation is lyophilized. Many different freeze-dryers are available for this purpose such as Hull50™ (Hull, USA) or GT20™ (Leybold-Heraeus, Germany) freeze-dryers. Freeze-drying is accomplished by freezing the formulation and subsequently subliming ice from the frozen content at a temperature suitable for primary drying. Under this condition, the product temperature is below the eutectic point or the collapse temperature of the formulation. Typically, the shelf temperature for the primary drying will range from about -30 to 25° C (provided the product remains frozen during primary drying) at a suitable pressure, ranging typically from about 50 to 250mTorr. The formulation, size and type of the container holding the sample (*e.g.*, glass vial) and the volume of liquid will mainly dictate the time required for drying, which can range from a few hours to several days (*e.g.* 40-60hrs). A secondary drying stage may be carried out at about 0-40° C, depending primarily on the type and size of container and the type of protein employed. However, it was found herein that a secondary drying step may not be necessary. For example, the shelf temperature throughout the entire water removal phase of lyophilization may be from about 15-30° C (*e.g.*, about 20° C). The time and pressure required for secondary drying will be that which produces a suitable lyophilized cake, dependent, *e.g.*, on the temperature and other parameters. The secondary drying time is dictated by the desired residual moisture level in the product and typically takes at least about 5 hours (*e.g.* 10-15 hours). The pressure may be the same as that employed during the primary drying step. Freeze-drying conditions can be varied depending on the formulation and vial size.

In some instances, it may be desirable to lyophilize the protein formulation in the container in which reconstitution of the protein is to be carried out in order to avoid a transfer step. The container in this instance may, for example, be a 3, 5, 10, 20, 50 or 100cc vial.

As a general proposition, lyophilization will result in a lyophilized formulation in which the moisture content thereof is less than about 5%, and preferably less than about 3%.

C. Reconstitution of the Lyophilized Formulation

At the desired stage, typically when it is time to administer the protein to the patient, the lyophilized formulation may be reconstituted with a diluent such that the protein concentration in the reconstituted formulation is at least 50 mg/mL, for example from about 50 mg/mL to about 400 mg/mL, more preferably from about 80 mg/mL to about 300 mg/mL, and most preferably from about 90 mg/mL to about 150 mg/mL. Such high protein concentrations in the reconstituted formulation are considered to be particularly useful where subcutaneous delivery of the reconstituted formulation is intended. However, for other routes of administration, such as intravenous administration, lower concentrations of the protein in the reconstituted formulation may be desired (for example from about 5-50 mg/mL, or from about 10-40 mg/mL protein in the reconstituted formulation). In certain embodiments, the protein concentration in the reconstituted formulation is significantly higher than that in the pre-lyophilized formulation. For example, the protein concentration in the reconstituted formulation may be about 2-40 times, preferably 3-10 times and most preferably 3-6 times (*e.g.* at least three fold or at least four fold) that of the pre-lyophilized formulation.

Reconstitution generally takes place at a temperature of about 25° C to ensure complete hydration, although other temperatures may be employed as desired. The time required for reconstitution will depend, *e.g.*, on the type of diluent, amount of excipient(s) and protein. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.* phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution. The diluent optionally contains a preservative. Exemplary preservatives have been described above, with aromatic alcohols such as benzyl or phenol alcohol being the preferred preservatives. The amount of preservative employed is determined by assessing different preservative concentrations for compatibility with the protein and preservative efficacy testing. For example, if the preservative is an aromatic alcohol (such as benzyl alcohol), it can be present in an amount from about 0.1-2.0% and preferably from about 0.5-1.5%, but most preferably about 1.0-1.2%.

Preferably, the reconstituted formulation has less than 6000 particles per vial which are ≥ 10 μm in size.

D. Administration of the Reconstituted Formulation

The reconstituted formulation is administered to a mammal in need of treatment with the protein, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

In preferred embodiments, the reconstituted formulation is administered to the mammal by subcutaneous (*i.e.* beneath the skin) administration. For such purposes, the formulation may be injected using a syringe. However, other devices for administration of the formulation are available such as injection devices (*e.g.* the Inject-ease™ and Genject™ devices); injector pens (such as the GenPen ™); needleless devices (*e.g.* MediJector™ and BioJector™); and subcutaneous patch delivery systems.

The appropriate dosage ("therapeutically effective amount") of the protein will depend, for example, on the condition to be treated, the severity and course of the condition, whether the protein is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the protein, the type of protein used, and the discretion of the attending physician. The protein is suitably administered to the patient at one time or over a series of treatments and may be administered to the patient at any time from

diagnosis onwards. The protein may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the condition in question.

Where the protein of choice is an antibody, from about 0.1-20 mg/kg is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques.

In the case of an anti-HER2 antibody, a therapeutically effective amount of the antibody may be administered to treat or prevent cancer characterized by overexpression of the HER2 receptor. It is contemplated that a reconstituted formulation of the anti-HER2 antibody may be used to treat breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon and/or bladder cancer. For example, the anti-HER2 antibody may be used to treat ductal carcinoma *in situ* (DCIS). Exemplary dosages of the anti-HER2 antibody are in the range 1-10 mg/kg by one or more separate administrations.

Uses for an anti-IgE formulation include the treatment or prophylaxis of IgE-mediated allergic diseases, parasitic infections, interstitial cystitis and asthma, for example. Depending on the disease or disorder to be treated, a therapeutically effective amount (*e.g.* from about 1-15 mg/kg) of the anti-IgE antibody is administered to the patient.

E. Articles of Manufacture

In another embodiment of the invention, an article of manufacture is provided which contains the lyophilized formulation of the present invention and provides instructions for its reconstitution and/or use. The article of manufacture comprises a container. Suitable containers include, for example, bottles, vials (*e.g.* dual chamber vials), syringes (such as dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the lyophilized formulation and the label on, or associated with, the container may indicate directions for reconstitution and/or use. For example, the label may indicate that the lyophilized formulation is reconstituted to protein concentrations as described above. The label may further indicate that the formulation is useful or intended for subcutaneous administration. The container holding the formulation may be a multi-use vial, which allows for repeat administrations (*e.g.* from 2-6 administrations) of the reconstituted formulation. The article of manufacture may further comprise a second container comprising a suitable diluent (*e.g.* BWFI). Upon mixing of the diluent and the lyophilized formulation, the final protein concentration in the reconstituted formulation will generally be at least 50 mg/mL. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature citations are incorporated by reference.

EXAMPLE 1

ANTI-HER2 FORMULATION

Overexpression of the *HER2* proto-oncogene product ($p185^{HER2}$) has been associated with a variety of aggressive human malignancies. The murine monoclonal antibody known as muMAb4D5 is directed against the extracellular domain (ECD) of $p185^{HER2}$. The muMAb4D5 molecule has been humanized in an attempt to improve its clinical efficacy by reducing immunogenicity and allowing it to support human effector functions

(see WO 92/22653). This example describes the development of a lyophilized formulation comprising full length humanized antibody huMAb4D5-8 described in WO 92/22653.

In the development of a lyophilized formulation, excipients and buffers are initially screened by measuring the stability of the protein after lyophilization and reconstitution. The lyophilized protein in each formulation is also subjected to accelerated stability studies to determine the potential stability of the protein over its shelf-life. These accelerated studies are usually performed at temperatures above the proposed storage conditions and the data are then used to estimate the activation energy for the degradation reactions assuming Arrhenius kinetics (Cleland *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems* 10(4): 307-377 (1993)). The activation energy is then used to calculate the expected shelf-life of the protein formulation at the proposed storage conditions.

In early screening studies, the stability of several lyophilized recombinant humanized anti-HER2 antibody (rhuMAb HER2) formulations was investigated after incubation at 5° C (proposed storage condition) and 40° C (accelerated stability condition). In the liquid state, rhuMAb HER2 was observed to degrade by deamidation (30Asn of light chain) and isoaspartate formation via a cyclic imide intermediate, succinimide (102Asp of heavy chain). The deamidation was minimized at pH 5.0 resulting in degradation primarily at the succinimide. At pH 6.0, slightly greater deamidation was observed in the liquid protein formulation. The lyophilized formulations were therefore studied with: (a) 5 or 10 mM succinate buffer, pH 5.0 or (b) 5 or 10 mM histidine buffer, pH 6.0. Both buffers contained the surfactant, polysorbate 20 (Tween 20™), which was employed to reduce the potential for aggregation of the reconstituted protein and minimize the formation of particulates after reconstitution. These buffers were used with and without various sugars. The protein was formulated in the buffer at 5.0, 21.0 or 25.0 mg/mL. These formulations were then lyophilized and assessed for protein stability after 2 weeks at 5° C and 40° C. In the lyophilizer, the vials were frozen at a shelf temperature of -55° C for approximately 5 hours followed by primary drying at a shelf temperature of 5° C and 150 mTorr for 30 hours, and drying to 1-2% residual moisture was achieved with secondary drying at a shelf temperature of 20° C for 10 hours. The major degradation route for this protein upon lyophilization was aggregation, and therefore the protein stability was assessed by native size exclusion chromatography to measure the recovery of intact native protein (% intact protein in Table 2 below).

The stabilizing effects of various lyoprotectant sugars on lyophilized protein was measured in 10 mM sodium succinate, pH 5.0 (Table 2). At high sugar concentrations (250-275 mM) and low protein concentration (5.0 mg/mL), trehalose and lactose stabilized the protein against aggregation for the lyophilized protein stored for 2 weeks at 40° C. However, lactose, a reducing sugar, was observed to react with the protein over longer term storage at 40° C. The formulations at 5.0 mg/mL protein containing either sorbitol or mannitol yielded aggregated protein after storage at 40° C for 2 weeks. At the higher protein concentration (21.0 mg/mL), formulations comprising mannitol, or mannitol in combination with sorbitol or glycine, contained aggregated protein after lyophilization and storage at both conditions. In contrast, trehalose and sucrose prevented aggregation at both storage conditions.

The 250 mM trehalose and 250 mM lactose formulations were assessed for long term stability. After 9 months at 40° C or 12 months at 5° C, there was no change in the % intact protein for the trehalose formulation. For the lactose formulation, the % intact protein remained constant (same as initial) after 3 months at 40° C or

6 months at 25° C. The trehalose formulation could be stored at controlled room temperature (15-30° C) for 2 years without a significant change in % intact protein.

The 10 mM histidine, pH 6.0 formulation with mannitol contained less aggregated protein after storage at 40° C for 2 weeks than the 10 mM succinate formulation, pH 5.0 with mannitol. This result may be related to some stabilizing effect contributed by histidine alone. After storage at 40° C for 2 weeks, there was, however, significant aggregation for histidine alone or histidine/mannitol formulations. The addition of sucrose at an equal mass to mannitol (10 mg/mL of each) in the histidine formulation stabilized the protein against aggregation for both storage conditions. The use of glycine with mannitol did not improve the protein stability, while the sucrose/glycine formulation provided the same stability as the sucrose/mannitol formulation. These results further indicated that sucrose was useful for preventing aggregation of the lyophilized protein during storage.

TABLE 2

Composition Prior to Lyophilization		% Intact Protein[a]		
[Protein][b] (mg/mL)	Formulation	Liquid (5° C)	Lyophilized (2 wk, 5° C)	Lyophilized (2wk, 40° C)
	10 mM sodium succinate pH 5.0			
5.0	275 mM trehalose, 0.01% Tween 20™	98.9	99.1	98.9
5.0	275 mM lactose, 0.01% Tween 20™	96.8	96.5	96.6
5.0	275 mM sorbitol, 0.01% Tween 20™	99.4	99.3	95.4
5.0	250 mM mannitol, 0.01% Tween 20™	100.0	99.9	98.8
5.0	250 mM trehalose, 0.01% Tween 20™	100.0	99.9	100.0
5.0	250 mM lactose, 0.01% Tween 20™	100.0	100.0	100.0
21.0	250 mM trehalose, 0.2% Tween 20™	99.3	99.1	99.1
21.0	250 mM sucrose, 0.2% Tween 20™	99.6	99.6	99.7
21.0	250 mM mannitol, 0.01% Tween 20™	100.0	94.6	94.0
21.0	188 mM mannitol/63 mM sorbitol, 0.01% Tween 20™	99.8	98.6	96.5
21.0	250 mM mannitol/25 mM glycine, 0.01% Tween 20™	99.5	96.5	96.4
	10 mM histidine pH 6.0			
21.0	No sugar, 0.01% Tween 20™	100.0	99.9	98.9
21.0	54.9 mM mannitol, 0.01% Tween 20™	100.0	99.9	99.2
21.0	29.2 mM sucrose/266.4 mM glycine, 0.01% Tween 20™	100.0	100.0	99.6
21.0	54.9 mM mannitol/266.4 mM glycine, 0.01% Tween 20™	100.0	99.8	98.9

Composition Prior to Lyophilization		% Intact Protein[a]		
[Protein][b] (mg/mL)	Formulation	Liquid (5° C)	Lyophilized (2 wk, 5° C)	Lyophilized (2wk, 40° C)
21.0	54.9 mM mannitol/29.2 mM sucrose, 0.01% Tween 20™	99.8	100.0	99.7

a. The fraction of intact protein was measured by native size exclusion HPLC and the peak area of the native protein relative to the total peak area including aggregates (TSK3000 SW XL column, TosoHaas, with a flow rate of 1.0 mL/min; elution with phosphate buffered saline; detection at 214 and 280 nm). The protein formulations were analyzed before lyophilization (liquid, 5° C) and after lyophilization and storage at 5° C or 40° C for 2 weeks.
b. Formulations containing 5 mg/mL protein were reconstituted with distilled water (20 mL, 5.0 mg/mL protein), and formulations containing 21 mg/mL protein were reconstituted with bacteriostatic water for injection (BWFI, 0.9% benzyl alcohol; 20 mL, 20 mg/mL protein).

The delivery of a high protein concentration is often required for subcutaneous administration due to the volume limitations (≤ 1.5 mL) and dosing requirements (≥100 mg). However, high protein concentrations (≥ 50 mg/mL) are often difficult to achieve in the manufacturing process since at high concentrations, the protein has a tendency to aggregate during processing and becomes difficult to manipulate (*e.g.* pump) and sterile filter. Alternatively, the lyophilization process may provide a method to allow concentration of the protein. For example, the protein is filled into vials at a volume (Vf) and then lyophilized. The lyophilized protein is then reconstituted with a smaller volume (Vr) of water or preservative (*e.g.* BWFI) than the original volume (*e.g.* Vr = 0.25Vf) resulting in a higher protein concentration in the reconstituted solution. This process also results in the concentration of the buffers and excipients. For subcutaneous administration, the solution is desirably isotonic.

The amount of trehalose in the lyophilized rhuMAb HER2 was reduced to produce an isotonic solution upon reconstitution to yield 100 mg/mL protein. The stabilizing effect of trehalose was determined as a function of concentration for 5 mM sodium succinate, pH 5.0 and 5 mM histidine, pH 6.0 at 25.0 mg/mL protein (Table 3). At trehalose concentrations from 60 to 200 mM, there was no significant aggregation after incubation of the lyophilized protein for 4 weeks at 40° C. These formulations were reconstituted with 20 mL of bacteriostatic water for injection (BWFI, USP, 0.9% benzyl alcohol). Reconstitution of the 50 mM trehalose formulation (5 mM sodium succinate) with 4 mL of BWFI (100 mg/mL protein) after incubation for 4 weeks at 40° C yielded a slight increase in aggregate formation. The preserved reconstituted formulations provided the advantage of multiple withdrawals from the same vial without sterility concerns. When sterile needles are used, these formulations would then allow for several doses from a single vial.

TABLE 3

Composition Prior to Lyophilization		% Intact Protein[a]		
[Protein] (mg/mL)	Formulation	Liquid (5° C)	Lyophilized (4 wk, 5° C)	Lyophilized (4 wk, 40° C)
	5 mM sodium succinate pH 5.0			
25.0	50 mM trehalose, 0.01% Tween 20™ [b]	100.00	100.0	99.5
25.0	60 mM trehalose, 0.01% Tween 20™	100.0	100.0	99.9
25.0	60 mM trehalose, 0.01% Tween 20™	100.0	100.0	99.2
25.0	100 mM trehalose, 0.01% Tween 20™	100.0	100.0	99.7
25.0	150 mM trehalose, 0.01% Tween 20™	100.0	100.0	99.8
25.0	200 mM trehalose, 0.01% Tween 20™	100.0	100.0	100.0
	5 mM histidine pH 6.0			
25.0	38.4 mM mannitol/20.4 mM sucrose, 0.01% Tween 20™	100.0	100.0	99.3
25.0	38.4 mM mannitol/20.4 mM sucrose, 0.01% Tween 20™ [c]	100.0	100.0	99.4
25.0	60 mM trehalose, 0.01% Tween 20™ [d]	100.0	100.0	99.8
25.0	60 mM trehalose, 0.01% Tween 20™	100.0	100.0	99.4
25.0	100 mM trehalose, 0.01% Tween 20™	100.0	100.0	99.6
25.0	150 mM trehalose, 0.01% Tween 20™	100.0	100.0	100.0
25.0	200 mM trehalose, 0.01% Tween 20™	100.0	100.0	100.0

a. The fraction of intact protein was measured by native size exclusion HPLC and defined as the peak area of the native protein relative to the total peak area including aggregates (TSK3000 SW XL column, TosoHaas, with a flow rate of 1.0 mL/min; elution with phosphate buffered saline; detection at 214 and 280 nm). The protein formulations were analyzed before lyophilization (liquid, 5° C) and after lyophilization and storage at 5° C or 40° C for 4 weeks. Formulations were reconstituted with bacteriostatic water for injection (BWFI, USP, 0.9% w/w benzyl alcohol; 20 mL, 22 mg/mL protein).

b. Reconstituted with 4 mL of BWFI (0.9% benzyl alcohol) to yield 100 mg/mL protein.

c. Reconstituted with 4 mL of BWFI (1.1% benzyl alcohol) to yield 100 mg/mL protein.

d. Sample incubated for 2 weeks at 5° C or 40° C and then reconstituted with 20 mL of BWFI (0.9% benzyl alcohol) to yield 22 mg/mL protein.

Currently, rhuMAb HER2 is under investigation as a therapeutic for the treatment of breast cancer. The protein is dosed to patients at 2 mg/kg on a weekly basis. Since the average weight of these patients is 65 kg, the average weekly dose is 130 mg of rhuMAb HER2. For subcutaneous administration, injection volumes of 1.5 mL or less are well tolerated and, therefore, the protein concentration for a weekly subcutaneous administration of rhuMAb HER2 may be approximately 100 mg/mL (130 mg average dose/1.5 mL). As mentioned above, this high protein concentration is difficult to manufacture and maintain in a stable form. To achieve this high protein concentration, rhuMAb HER2 formulated in: (a) 5 mM sodium succinate, pH 5.0 or (b) 5 mM histidine, pH 6.0, was lyophilized at 25 mg/mL protein in 60 mM trehalose, 0.01% Tween 20™. The lyophilization was performed by filling 18 mL of the protein formulation into 50 cc vials. In the lyophilizer, the vials were frozen at a shelf

temperature of -55° C for approximately 5 hours followed by primary drying at a shelf temperature of 5° C and 150 mTorr for 30 hours, and drying to 1-2% residual moisture was achieved with secondary drying at a shelf temperature of 20° C for 10 hours. Thermocouples placed in vials containing the placebo (formulation without protein) indicated that the product in the vials was maintained below -10° C throughout primary drying. Sequential stoppering studies during the lyophilization revealed that the residual moisture after primary drying was usually less than 10%.

The lyophilized protein was then reconstituted with either 4 or 20 mL of BWFI (0.9 or 1.1% benzyl alcohol) to yield concentrated protein solutions:

(a) 4 mL: 102 mg/mL rhuMAb HER2, 245 mM trehalose, 21 mM sodium succinate, pH 5.0 or 21 mM histidine, pH 6.0, 0.04% Tween 20™;

(b) 20 mL: 22 mg/mL rhuMAb HER2, 52 mM trehalose, 4 mM sodium succinate, pH 5.0 or 4 mM histidine, pH 6.0, 0.009% Tween 20™.

After storage of the lyophilized formulations for 4 weeks at 40° C and reconstitution to 22 mg/mL protein, the amount of aggregated protein appeared to increase slightly with decreasing trehalose concentration. The stability of the lyophilized protein was not affected by the volume of reconstitution. As shown in Figure 1, the amount of intact protein after incubation of the lyophilized protein at 40° C was the same for the 60 mM trehalose, 5 mM sodium succinate, pH 5.0, 0.01% Tween 20™ formulation reconstituted with either 4 or 20 mL of BWFI.

The results shown in Table 3 suggested that there may be a relationship between the trehalose concentration and the protein stability. To further assess this relationship, the formulations containing different concentrations of trehalose formulated in either sodium succinate or histidine were incubated for 91 days at 40° C. The stability was then measured as a function of the trehalose to protein molar ratio for each concentration of trehalose. As shown in Figure 2, the protein stability clearly decreased with decreasing trehalose concentration for both formulations. There was no apparent difference between the two buffers, succinate and histidine, in these formulations suggesting that the primary stabilizer under these conditions is trehalose. In addition, the observed decrease in intact protein for both these formulations would be acceptable even at the low trehalose concentration for a formulation that is stored at 2-8° C throughout its shelf-life. However, if controlled room temperature (30° C maxmimum temperature) stability is required, higher trehalose concentrations (≥ 600:1 trehalose:protein) may be needed depending on the stability specifications for the product (*i.e.* the specification for the amount of intact protein remaining after 2 years of storage). Typically, a controlled room temperature storage condition would require stability for 6 months at 40° C which is equivalent to storage at 30° C for 2 years.

As shown in Figure 3, the 250 mM trehalose formulation was unchanged after 6 months at 40° C while both the 60 mM trehalose formulations were less stable. The 60 mM trehalose formulations may then require refrigerated storage if the product specification at the end of its shelf-life is, for example, >98% intact protein by native size exclusion chromatography.

In the previous screening study, sucrose was also observed to prevent aggregation of rhuMAb HER2 after lyophilization and subsequent storage. To achieve isotonic solutions after reconstitution for subcutaneous administration (approximately four-fold concentration of formulation components and protein), the sucrose concentration must be reduced significantly. The equal mass concentration of sucrose and mannitol (bulking

agent) used in the screening studies prevented aggregation of the protein. A lower concentration of sucrose and mannitol (equal mass concentrations) was chosen as a potential subcutaneous formulation of rhuMAb HER2. The protein solution (25 mg/mL protein, 5 mM histidine, pH 6.0, 38.4 mM (7 mg/mL) mannitol, 20.4 mM (7 mg/mL) sucrose, 0.01% Tween 20™) was lyophilized in the same manner as the 60 mM trehalose formulation except that the primary drying cycle was extended to 54 hours. After 4 weeks at 40°C, there was a slight increase in the amount of aggregates after reconstitution with 4.0 or 20.0 mL of BWFI (Table 3). The amount of aggregated protein was the same for reconstitution at 22 or 100 mg/mL protein (Figure 4). Like the 60 mM trehalose formulations, the mannitol/sucrose formulation yielded less intact protein over time at 40°C. The molar ratio of sucrose to protein for this formulation was 120 to 1, indicating that the mannitol/sucrose combination may be more effective than trehalose alone at the same molar ratio of stabilizing sugar (Figures 2 and 4).

In the previous examples, the stability of the lyophilized rhuMAb HER2 formulations was determined as a function of temperature. These studies demonstrated that the trehalose and mannitol/sucrose formulations prevented degradation of the protein in the lyophilized state at high temperatures (40°C). However, these experiments did not address the stability of the protein after reconstitution and storage. Once reconstituted with BWFI, the lyophilized rhuMAb HER2 formulations may be used for several administrations of the drug. In particular, the vial configuration (450 mg rhuMAb HER2) was designed to provide three doses to the average patient (130 mg rhuMAb HER2 per dose). Since the drug is dosed weekly, the vial may be stored at least three weeks after reconstitution. To assure that the rhuMAb HER2 remained stable after reconstitution, stability studies on the reconstituted rhuMAb HER2 formulations were performed at 5°C and 25°C.

For subcutaneous administration, the formulations were reconstituted to 100 mg/mL (4 mL BWFI). At this high protein concentration, the protein may be more susceptible to aggregation than the intravenous dosage form that was reconstituted to 22 mg/mL protein (20 mL BWFI). The four rhuMAb HER2 formulations from the previous example were assessed for aggregation (loss of intact protein). As shown in Tables 4 through 6, there was no difference in stability for formulations reconstituted at 22 and 100 mg/mL protein. Furthermore, these formulations maintained the protein completely intact for up to 90 days at 5 °C and 30 days at 25 °C, indicating that the reconstituted protein could be stored refrigerated for at least 90 days. Unlike the lyophilized protein stability in the previous example, the trehalose concentration in the formulation did not affect the protein stability (Table 7).

TABLE 4

Stability of the reconstituted formulations for rhuMAb HER2 lyophilized at 25 mg/mL protein in 5 mM sodium succinate, pH 5.0, 60 mM trehalose, 0.01% Tween 20™

Time (days)	% Intact Protein			
	22 mg/mL protein		100 mg/mL protein	
	5°C	25°C	5°C	25°C
0	99.9	99.9	99.7	99.7
14	ND	100.0	ND	100.0
30	100.0	100.0	100.0	100.0
91	99.8	ND	100	ND

The samples were reconstituted with 4.0 or 20.0 mL of BWFI (1.1% or 0.9% benzyl alcohol), and then stored at 5°C or 25°C. The % intact protein was defined as the fraction of native peak area as measured by native size exclusion chromatography. ND = not determined.

TABLE 5

Stability of the reconstituted formulations for rhuMAb HER2 lyophilized at 25 mg/mL protein in 5 mM histidine, pH 6.0, 60 mM trehalose, 0.01% Tween 20™

Time (days)	% Intact Protein			
	22 mg/mL protein		100 mg/mL protein	
	5°C	25°C	5°C	25°C
0	100.0	100.0	100.0	100.0
14	ND	100.0	ND	100.0
31	99.3	99.7	100.0	100.0
61	100.0	ND	ND	ND

The samples were reconstituted with 4.0 or 20.0 mL of BWFI (1.1% or 0.9% benzyl alcohol), and then stored at 5°C or 25 °C. The % intact protein was defined as the fraction of native peak area as measured by native size exclusion chromatography. ND = not determined.

TABLE 6

Stability of the reconstituted formulations for rhuMAb HER2 lyophilized at 25 mg/mL protein in 5 mM histidine, pH 6.0, 38.4 mM mannitol, 20.4 mM sucrose, 0.01% Tween 20™

Time (days)	% Intact Protein			
	22 mg/mL protein		100 mg/mL protein	
	5°C	25°C	5°C	25°C
0	99.7	99.7	99.8	99.8
14	ND	100.0	ND	99.8
31	100.0	100.0	100.0	100.0
92	100.0	ND	100.0	ND

The samples were reconstituted with 4.0 or 20.0 mL of BWFI (1.1% or 0.9% benzyl alcohol), and then stored at 5°C or 25 °C. The % intact protein was defined as the fraction of native peak area as measured by native size exclusion chromatography. ND = not determined.

TABLE 7

Stability of the reconstituted formulations for rhuMAb HER2 lyophilized at 21 mg/mL protein in 10 mM sodium succinate, pH 5.0, 250 mM trehalose, 0.2% Tween 20™

Time (days)	% Intact Protein 21 mg/mL protein	
	5°C	25°C
0	99.8	99.8
14	ND	100.0
31	99.9	99.4
92	99.8	ND

The samples were reconstituted with 20.0 mL of BWFI (0.9% benzyl alcohol), and then stored at 5°C or 25°C. The % intact protein was defined as the fraction of native peak area as measured by native size exclusion chromatography. ND = not determined.

As mentioned previously, the major degradation route for rhuMAb HER2 in aqueous solutions is deamidation or succinimide formation. The loss of native protein due to deamidation or succinimide formation was assessed for the four reconstituted rhuMAb HER2 formulations.

Analysis of rhuMAb HER2 deamidation and succinimide formation was performed using cation exchange chromatography. A Bakerbond Wide-Pore Carboxy Sulfon (CSX) column (4.6 x 250 mm) was operated at a flow rate of 1 mL/min. The mobile phase buffers were (A) 0.02 M sodium phosphate, pH 6.9, and (B) 0.02 M sodium phosphate, pH 6.9, 0.2 M NaCl. The chromatography was then performed at 40° C as follows:

TABLE 8

Time (min)	% Buffer B
0	10
55	45
57	100
62	100
62.1	10
63	10

Peak elution was monitored at 214 nm and 75 µg of protein was loaded for each analysis.

Again, there were no differences in stability of the formulations reconstituted at 22 and 100 mg/mL protein (Figures 5 through 7). The protein degradation was more rapid at 25° C than 5° C for each formulation, and the rate of degradation was comparable for all the formulations stored at 5° C. The formulations containing histidine underwent a slightly greater rate of degradation at 25° C than the succinate formulations. The amount of trehalose in the formulation did not affect the degradation rate at either temperature (Figures 5 and 8). These

results indicated that these four formulations provide an acceptable rate of degradation under refrigerated storage conditions (5° C) for the intended period of use (30 days after reconstitution with BWFI).

Multi-use formulations should pass preservative efficacy testing as described by the US Pharmacopeia (USP) for use in the United States. The rhuMAb HER2 lyophilized formulation consisting of 25 mg/mL protein, 5 mM histidine, pH 6.0, 60 mM trehalose, 0.01% Tween 20™ was reconstituted with 20 mL of benzyl alcohol at concentrations between 0.9 and 1.5% w/w. For concentrations at or above 1.3% w/w, the reconstituted solution became cloudy after overnight incubation at room temperature (~25 °C). Reconstitution with the standard BWFI solution (0.9% benzyl alcohol) resulted in a solution that did not consistently pass the preservative challenge tests. However, reconstitution with 1.0 or 1.1% benzyl alcohol was both compatible with the formulation and passed the preservative challenge testing. The manufacturer's specifications for the solution required a range of ± 10%, and therefore, the lyophilized formulations are reconstituted with 1.1% benzyl alcohol (1.1 ± 0.1%).

A single step lyophilization cycle for the rhuMAb HER2 formulation was developed . In the single step lyophilization cycle, rhuMAb HER2 at 25 mg/mL, 60 mM trehalose, 5 mM histidine pH 6 and 0.01% polysorbate 20 was lyophilized at a shelf temperature of 20° C. and a pressure of 150 mTorr. After 47 hours, the residual moisture content of the lyophilized cake was less than 5%. This lyophilization cycle is considered to be useful in that it simplifies the manufacturing process, by eliminating the secondary drying step.

EXAMPLE 2

ANTI-IgE FORMULATION

IgE antibodies bind to specific high-affinity receptors on mast cells, leading to mast cell degranulation and release of mediators, such as histamine, which produce symptoms associated with allergy. Hence, anti-IgE antibodies that block binding of IgE to its high-affinity receptor are of potential therapeutic value in the treatment of allergy. These antibodies must also not bind to IgE once it is bound to the receptor because this would trigger histamine release. This example describes the development of a lyophilized formulation comprising full length humanized anti-IgE antibody MaE11 described in Presta *et al. J. Immunology,* 151: 2623-2632 (1993).

Materials: Highly purified rhuMAb E25 (recombinant humanized anti-IgE antibody MaE11) which did not contain Tween 20™ was used in the formulations described below. Spectra/Por 7 dialysis membranes were purchased from Spectrum (Los Angeles, CA). All other reagents used in this study were obtained from commercial sources and were of analytical grade. Formulation buffers and chromatography mobile phase were prepared by mixing the appropriate amount of buffer and salt with Milli-Q water in a volumetric flask.

Formulation: E25 S Sepharose pool was dialyzed into formulation buffers as specified. Dialysis was accomplished by a minimum of 4 x 2L buffer exchanges over a 48 hour period at 2-8° C. Following dialysis, lyoprotectant was added at a isotonic concentration to some of the formulations as required. Protein concentration following dialysis was determined by UV spectroscopy using a molar absorptivity of 1.60. The dialyzed protein was diluted to the predetermined formulation concentration with an appropriate formulation buffer, sterile filtered using a 0.22 µm Millex-GV filter (Millipore) and dispensed into pre-washed and autoclaved glass vials. The vials were fitted with siliconized teflon lyophilization stoppers and lyophilized using the following conditions: the E25 formulation was frozen to -55° C at 80° C/hour and the vial content was kept frozen for 4 hours. The shelf temperature was ramped to 25° C at 10° C/hour for primary drying. Primary

drying was carried out at 25° C, 50 μ chamber vacuum pressure for 39 hours such that the residual moisture of the lyophilized cake was 1-2%. Following lyophilization, a vial of each formulation was removed for t=0 analysis and the remaining vials were held at various temperatures which include -70° C, 2-8° C, 25° C, 30° C (controlled room temperature) 40° C and 50° C.

Chromatography: Native size exclusion chromatography was carried out on a Bio-Rad Bio-Select™ SEC 250-5 column (300 x 7.8 mm). The column was equilibrated and ran in PBS at a flow rate of 0.5 mL/min using a Hewlett Packard 1090L HPLC equipped with a diode array detector. Molecular weight standards (Bio-Rad, Inc.) consisting of thyroglobulin (670 kd), gamma-globulin (158 kd), ovalbumin (44 kd), and cyanocobalamin (1.35 kd) were used to calibrate the column. The sample load was 25 μg and protein was detected by monitoring the UV absorption at 214 nm using Turbochrom 3 software (PE Nelson, Inc).

Hydrophobic Interaction Chromatography: $F(ab')_2$ fragments of the E25 antibody were chromatographed using a TosoHaas Butyl-NPR column (3.5 x 4.6 mm) and a Hewlett Packard 1090L HPLC equipped with a diode array detector. Elution buffer A was: 20 mM Tris, 2 M ammonium sulfate, 20% (v/v) glycerol, pH 8.0 while elution buffer B was: 20 mM Tris, 20% (v/v) glycerol, pH 8.0. The column was equilibrated with 10% elution buffer B at a flow rate of 1.0 mL/min for a minimum of 20 minutes. The sample load was 5 μg and protein was detected by monitoring the UV absorption at 214 nm using Turbochrom 3 data acquisition software (PE Nelson, Inc). Following injection of the sample, the column was maintained at 10% buffer B for 1 minute followed by a linear gradient of from 10% to 62% buffer B in 20 minutes. The column was washed with 100% buffer B for 5 minutes and re-equilibrated with 10% buffer B for a minimum of 20 minutes between successive sample injections.

Antibody Binding Activity: IgE receptor binding inhibition assay (IE25:2) was carried out as described in Presta *et al.*, *supra*, on samples diluted to 20 μg/mL and 30 μg/mL in assay diluent (phosphate buffered saline, 0.5% BSA, 0.05% polysorbate 20, 0.01% Thimerosol). Each dilution was then assayed in triplicate and the results were multiplied by an appropriate dilution factor to yield an active concentration. The results from 6 assays were averaged. The assay measures the ability of rhuMAb E25 to competitively bind to IgE and thereby prevent IgE from binding to its high affinity receptor which is immobilized to an ELISA plate. The results are divided by the antibody concentration as determined by UV absorption spectroscopy and reported as a specific activity. Previous experiments have shown that this assay is stability indicating.

Particulate Assay: Reconstituted vials of lyophilized rhuMAb E25 were pooled to achieve a volume of approximately 7 mL. A count of the number of particles of size ranging from 2 to 80 μm present in 1 mL of sample was determined using a Hiac/Royco model 8000 counter. The counter was first washed with 1 mL of sample three times followed by the measurement of 1 mL of sample in triplicate. The instrument determines the number of particles per mL that are equal to or greater than 10 μm and the number of particles per mL that are equal to or greater than 25 μm.

The first step in the development of a formulation for the anti-IgE antibody was to determine a suitable buffer and pH for lyophilization and storage of the product. Antibody at a concentration of 5.0 mg/mL was formulated into 10 mM succinate buffers ranging from pH 5.0 to pH 6.5 and into sodium phosphate, potassium phosphate and histidine buffers at pH 7.0. Figure 9 shows increased antibody aggregate was observed in the higher pH formulations both before and after lyophilization. An exception was the histidine formulation at pH

7, where no increase in aggregate was observed upon storage at 2-8° C. Figure 10 shows rhuMAb E25 lyophilized in 5 mM histidine buffer at both pH 6 and pH 7 and stored for 1 year at 2-8° C, 25° C, and 40° C. At each assay time point and storage temperature the pH 6 formulation had less aggregate than the antibody formulated at pH 7. These results show histidine at pH 6 is a particularly useful buffer system for preventing aggregation of the antibody.

To facilitate screening of lyoprotectants, the anti-IgE antibody was formulated into sodium succinate at pH 5 with or without a lyoprotectant. Potential lyoprotectants, added at isotonic concentrations, were grouped into 3 categories:

(a) non-reducing monosaccharide (*i.e.* mannitol);

(b) reducing disaccharides (*i.e.* lactose and maltose); and

(c) non-reducing disaccharides (*i.e.* trehalose and sucrose).

Aggregation of the formulations following storage at 2-8° C and 40° C for one year is shown in Figures 11 and 12. With storage at 2-8° C, the monosaccharide formulation (mannitol) aggregated at a rate similar to the buffer control, while formulations containing the disaccharides were equally effective in controlling aggregation (Figure 11). The results following storage at 40° C where similar with the exception of the sucrose formulation which rapidly aggregated (which correlated with a browning of the freeze-dried cake (Figure 12)). This was later shown to be caused by degradation of sucrose following storage at both acidic pH and high temperature.

Hydrophobic interaction chromatography of the antibody formulated in histidine buffer at pH 6 with lactose shows the antibody is altered following storage for 6 months at 40° C (Figure 13). The chromatography peaks are broadened and the retention time decreases. These changes are not observed with the buffer control and sucrose formulations stored under similar conditions as shown in Figures 14 and 15, respectively. Furthermore, isoelectric focusing showed an acidic shift in the pI of the antibody formulated in lactose and stored at 25° C and 40° C. This indicates that reducing sugars are not suitable as lyoprotectants for the antibody.

Aggregation of lyophilized formulations of anti-IgE at a concentration of 20 mg/mL in 5 mM histidine buffer at pH 6 with various concentrations of sucrose and trehalose following storage for 12 weeks at 50° C is shown in Figure 16. Both sugars have a similar protective effect on aggregation when the sugar concentration is greater than 500 moles of sugar per mole of antibody. From these results, isotonic and hypertonic formulations of both sucrose and trehalose were identified for further development. The formulations are designed to be filled prior to lyophilization at a relatively low concentration of antibody and the lyophilized product is reconstituted with less volume than was filled with bacteriostatic water for injection (BWFI) comprising 0.9% benzyl alcohol. This allows the concentration of the antibody immediately prior to subcutaneous delivery and includes a preservative for a potential multi-use formulation while avoiding interactions between the protein and preservative upon long-term storage.

Isotonic formulation: Anti-IgE at 25 mg/mL formulated in 5 mM histidine buffer at pH 6 with 500 moles of sugar per mole antibody which equals a sugar concentration of 85 mM. This formulation is reconstituted with BWFI (0.9% benzyl alcohol) at a volume which is four times less than was filled. This results in a 100 mg/mL of antibody in 20 mM histidine at pH 6 with an isotonic sugar concentration of 340 mM.

Hypertonic formulation: Anti-IgE at 25 mg/mL formulated in 5 mM histidine buffer at pH 6 with 1000 moles of sugar per mole antibody which equals a sugar concentration of 161 mM. This formulation is reconstituted with BWFI (0.9% benzyl alcohol) at a volume which is four times less than was filled. This results in a 100 mg/mL of antibody in 20 mM histidine at pH 6 with a hypertonic sugar concentration of 644 mM.

Comparisons of the antibody aggregation following storage of the isotonic and hypertonic formulations for up to 36 weeks are shown in Figures 17 to 19. No change in aggregation is observed in either the hypertonic or isotonic formulations with storage at 2-8° C (Figure 17). With storage at controlled room temperature (30° C) increased aggregation is not observed in the hypertonic formulations while an increase in aggregate of from 1 to 2% occurs in the isotonic formulations (Figure 18). Finally, following storage at 50° C a minimal increase in aggregate is observed with the hypertonic formulations, a 4% increase in aggregate occurs with the isotonic trehalose formulation and a 12% increase in aggregate occurs with the isotonic sucrose formulation (Figure 19). These results show the isotonic formulation contains the minimum amount of sugar necessary to maintain the stability of the antibody with storage at a temperature up to 30° C.

The binding activity of the anti-IgE in the isotonic and hypertonic formulations was measured in an IgE receptor inhibition assay. It was discovered that the binding activity of the isotonic and hypertonic sucrose and trehalose formulations was essentially unchanged following storage at -70° C, 2-8° C, 30° C and 50° C for up to 36 weeks.

Lyophilized formulations of proteins are known to contain insoluble aggregates or particulates (Cleland *et al., Critical Reviews in Therapeutic Drug Carrier Systems*, 10 (4):307-377 (1993)). Accordingly, a particulate assay of antibody lyophilized at a concentration of 25 mg/mL in 5 mM histidine, pH 6 with the addition of 85 mM and 161 mM sucrose and trehalose was performed. Polysorbate 20 was added to the formulations at a concentration of 0.005%, 0.01%, and 0.02%. Samples were lyophilized and assayed following reconstitution to 100 mg/mL antibody in 20 mM histidine, pH 6 with 340 mM and 644 mM sugar. The polysorbate 20 concentration following reconstitution was 0.02%, 0.04%, and 0.08%.

Table 9 below shows the number of particles of size equal to or greater than 10 μm and equal to or greater than 25 μm from the isotonic and hypertonic sucrose and trehalose formulations. Polysorbate 20 was added to the formulations at concentrations of 0.005%, 0.01%, and 0.02% prior to lyophilization. The results show that the addition of Tween™ to the formulation significantly reduces the number of particles in each size range tested. The US Pharmacopeia (USP) specification for small volume injections are not more than 6,000 particles of greater than or equal to 10 μm and not more than 600 particles of greater than or equal to 25 μm per container (Cleland *et al., supra*). With the addition of polysorbate 20, both the hypertonic and isotonic formulations pass this specification.

TABLE 9

Formulation	Polysorbate 20	Particles per mL	
		≥ 10 µm	≥ 25 µM
Isotonic Sucrose	None	16,122	28
	0.005%	173	2
	0.01%	224	5
	0.02%	303	6
Hypertonic Sucrose	None	14,220	84
	0.005%	73	6
	0.01%	51	0
	0.02%		6
Isotonic Trehalose	None	33,407	24
	0.005%	569	4
	0.01%	991	16
	0.02%	605	9
Hypertonic Trehalose	None	24,967	28
	0.005%	310	11
	0.01%	209	6
	0.02%	344	6

One formulation developed for the anti-IgE antibody (*i.e.* 143 mg vial isotonic formulation of rhuMAb E25) which is considered to be useful for subcutaneous delivery of this antibody is shown in Table 10 below. A 10 cc vial is filled with 5.7 mL of rhuMAb E25 at a concentration of 25 mg/mL formulated in 5 mM histidine at pH 6.0 with 0.01% polysorbate 20. Sucrose is added as a lyoprotectant at a concentration of 85 mM which corresponds to a molar ratio of sugar to antibody of 500 to 1. The vial is lyophilized and reconstituted with 0.9% benzyl alcohol to one quarter of the volume of the fill or 1.2 mL. The final concentration of components in the formulation is increased four fold to 100 mg/mL rhuMAb E25 in 20 mM histidine at pH 6 with 0.04% polysorbate 20 and 340 mM sucrose (isotonic) and 0.9% benzyl alcohol. The formulation contains histidine buffer at pH 6 because of its demonstrated protective effect on antibody aggregation. Sucrose was added as the lyoprotectant because of previous use in the pharmaceutical industry. The concentration of sugar was chosen to result in an isotonic formulation upon reconstitution. Finally, polysorbate 20 is added to prevent the formation of insoluble aggregates.

TABLE 10

Pre-lyophilized Formulation (Fill 5.7 mL into 10 cc vial)	Reconstituted Formulation (1.2 mL 0.9% Benzyl Alcohol)
25 mg/mL rhuMAb E25	100 mg/mL rhuMAb E25
5 mM Histidine, pH 6.0	20 mM Histidine, pH 6.0
85 mM Sucrose	340 mM Sucrose
0.01% Polysorbate 20	0.04% Polysorbate 20
-	0.9% Benzyl Alcohol

WHAT IS CLAIMED IS:

1. A stable isotonic reconstituted formulation comprising a protein in an amount of at least about 50 mg/mL and a diluent, which reconstituted formulation has been prepared from a lyophilized mixture of a protein and a lyoprotectant, wherein the protein concentration in the reconstituted formulation is about 2-40 times greater than the protein concentration in the mixture before lyophilization.

2. The formulation of claim 1 wherein the lyoprotectant is sucrose or trechalose.

3. The formulation of claim 1 which further comprises a buffer.

4. The formulation of claim 3 wherein the buffer is histidine or succinate.

5. The formulation of claim 1 which further comprises a surfactant.

6. A stable reconstituted formulation comprising an antibody in an amount of at least about 50 mg/mL and a diluent, which reconstituted formulation has been prepared from a lyophilized mixture of an antibody and a lyoprotectant, wherein the antibody concentration in the reconstituted formulation is about 2-40 times greater than the antibody concentration in the mixture before lyophilization.

7. The formulation of claim 6 wherein the antibody is an anti-IgE antibody or anti-HER2 antibody.

8. The formulation of claim 6 which is isotonic.

9. A method for preparing a stable isotonic reconstituted formulation comprising reconstituting a lyophilized mixture of a protein and a lyoprotectant in a diluent such that the protein concentration in the reconstituted formulation is at least 50 mg/mL, wherein the protein concentration in the reconstituted formulation is about 2-40 times greater than the protein concentration in the mixture before lyophilization.

10. A method for preparing a formulation comprising the steps of:

(a) lyophilizing a mixture of a protein and a lyoprotectant; and

(b) reconstituting the lyophilized mixture of step (a) in a diluent such that the reconstituted formulation is isotonic and stable and has a protein concentration of at least about 50 mg/mL.

11. The method of claim 10 wherein the protein concentration in the reconstituted formulation is from about 80 mg/mL to about 300 mg/mL.

12. The method of claim 10 wherein the protein concentration in the reconstituted formulation is about 2-40 times greater than the protein concentration in the mixture before lyophilization.

13. The method of claim 10 wherein lyophilization is performed at a shelf temperature maintained at about 15-30° C throughout the entire lyophilization process.

14. An article of manufacture comprising:

(a) a container which holds a lyophilized mixture of a protein and a lyoprotectant; and

(b) instructions for reconstituting the lyophilized mixture with a diluent to a protein concentration in the reconstituted formulation of at least about 50 mg/mL.

15. The article of manufacture of claim 14 further comprising a second container which holds a diluent.

16. The article of manufacture of claim 15 wherein the diluent is bacteriostatic water for injection (BWFI) comprising an aromatic alcohol.

17. A formulation comprising a lyophilized mixture of a lyoprotectant and an antibody, wherein the molar ratio of lyoprotectant:antibody is about 100-1500 mole lyoprotectant:1 mole antibody.

18. Use of the formulation of claim 1 in the preparation of a medicament for treating a mammal which has a disorder requiring treatment with the protein in the formulation.

19. Use as in claim 18 wherein the formulation is for subcutaneous administration.

20. A formulation comprising anti-HER2 antibody in amount from about 5-40 mg/mL, sucrose or trehalose in an amount from about 10-100 mM, a buffer and a surfactant.

21. The formulation of claim 20 further comprising a bulking agent.

22. The formulation of claim 20 which is lyophilized and stable at 30° C for at least 6 months.

23. The formulation of claim 20 which is reconstituted with a diluent such that the anti-HER2 antibody concentration in the reconstituted formulation is from about 10-30 mg/mL, wherein the reconstituted formulation is stable at 2-8° C for at least about 30 days.

24. A formulation comprising anti-IgE antibody in amount from about 5-40 mg/mL, sucrose or trehalose in an amount from about 80-300 mM, a buffer and a surfactant.

25. The formulation of claim 24 which is lyophilized and stable at about 30° C for at least 1 year.

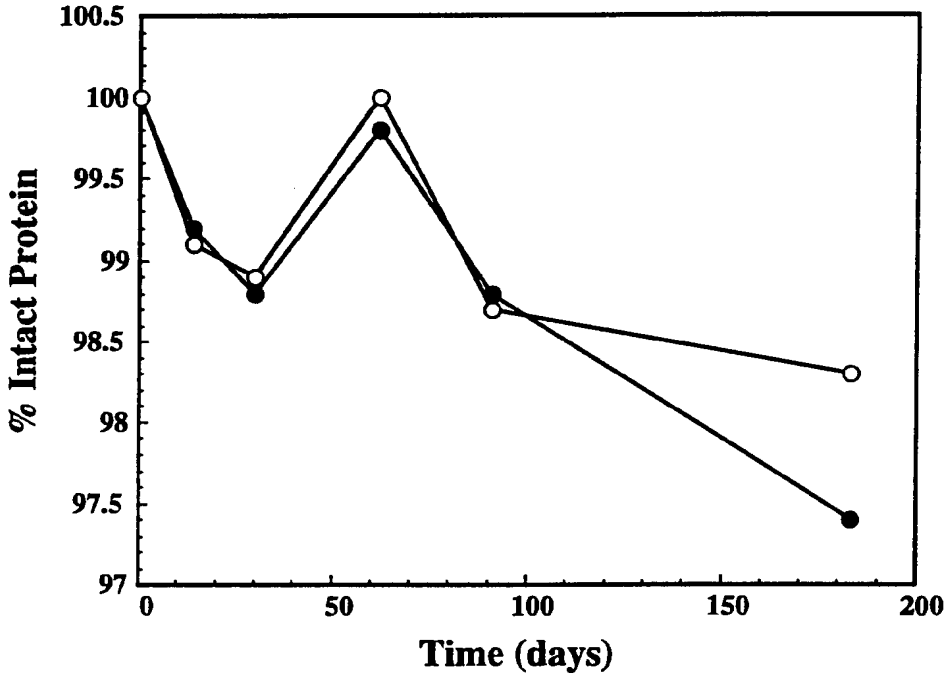


FIG. 1

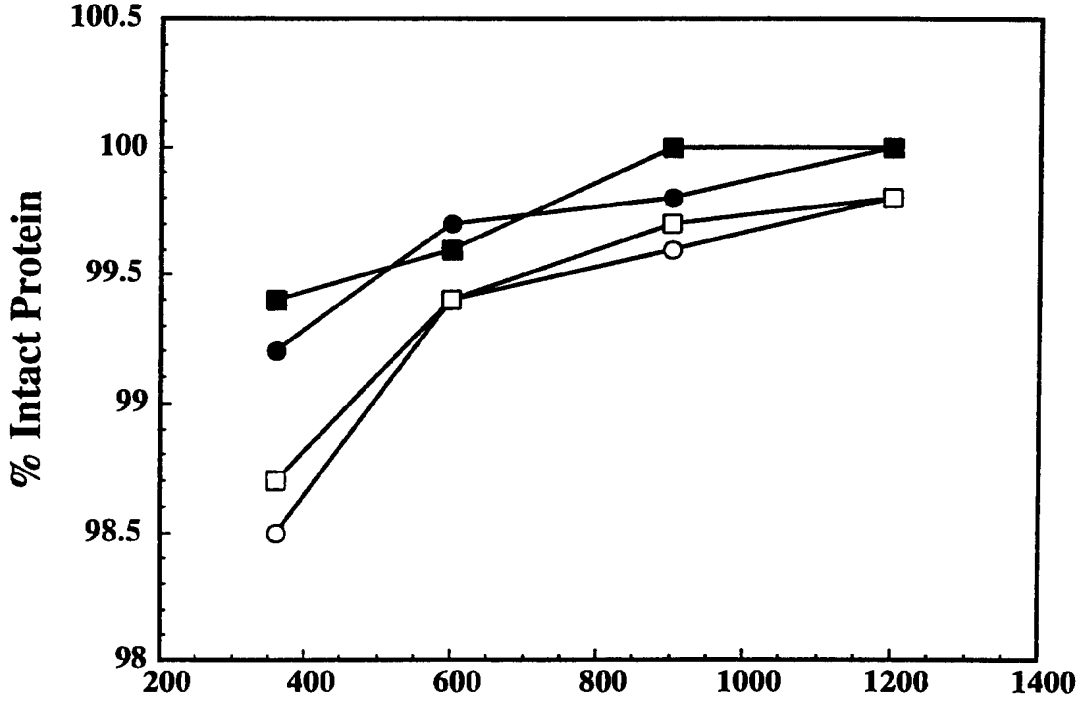


FIG. 2

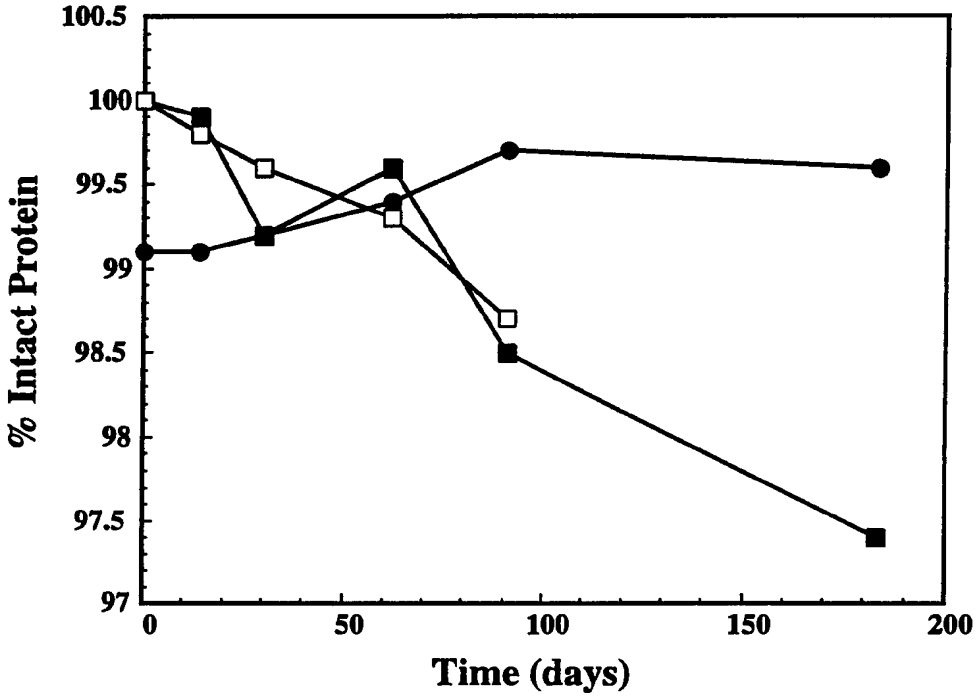


FIG. 3

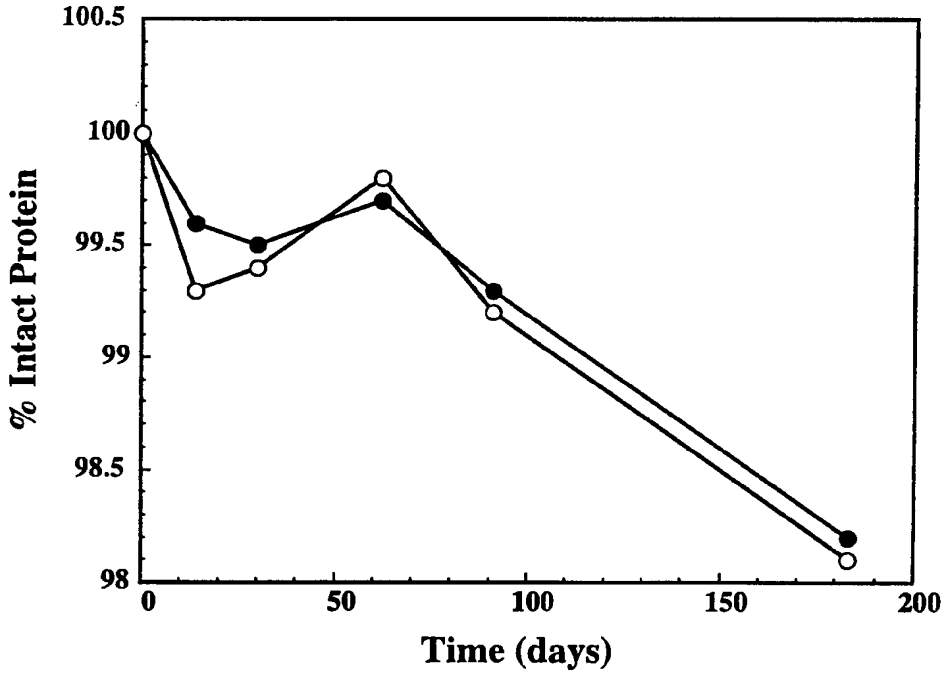


FIG. 4

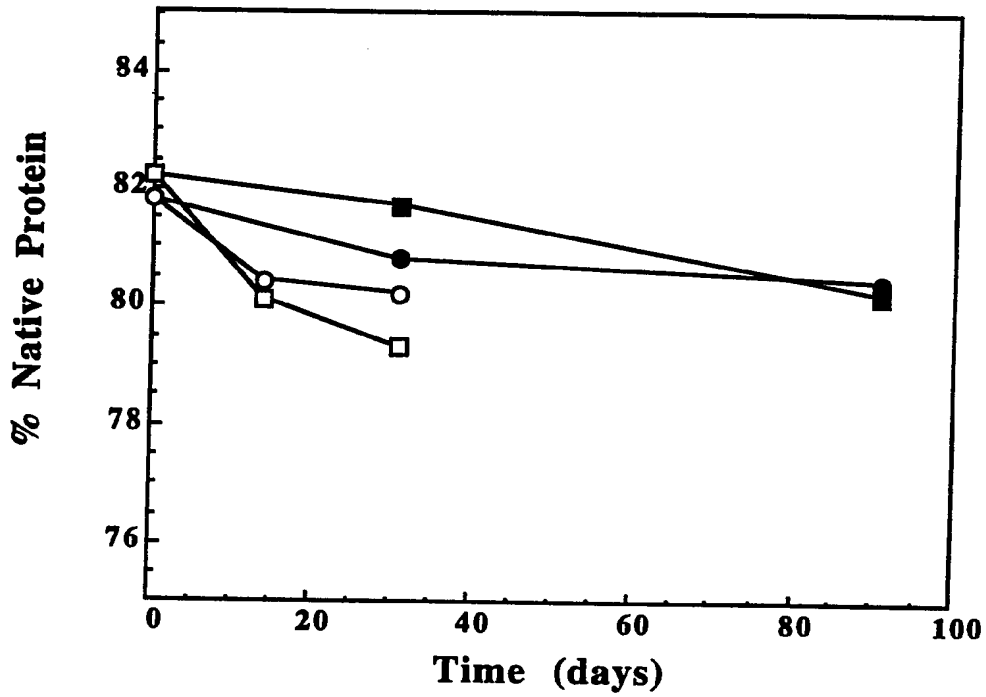


FIG. 5

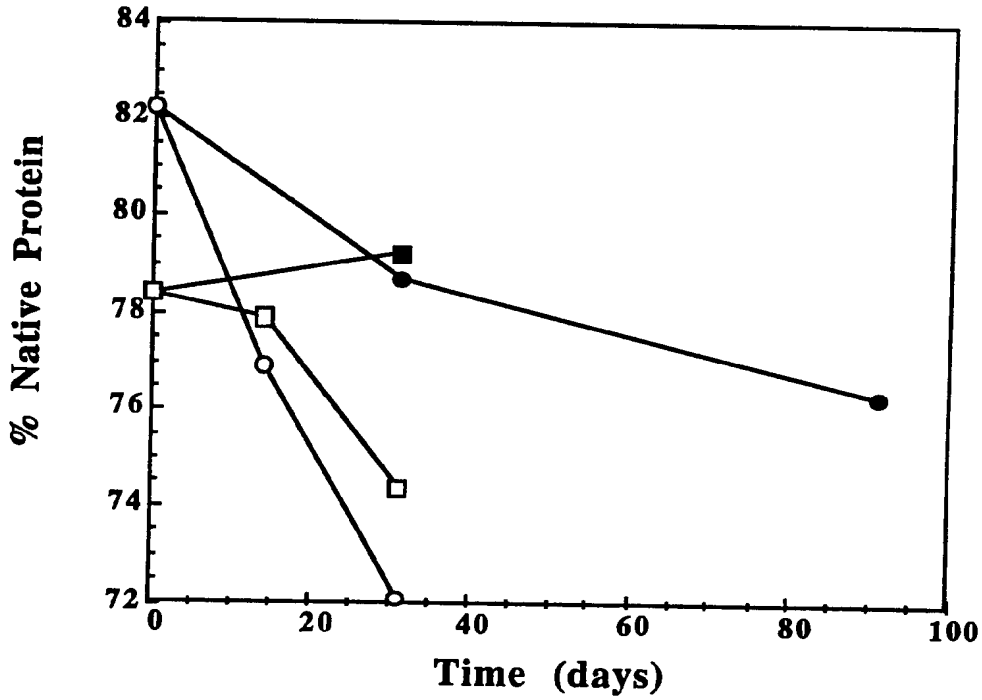


FIG. 6

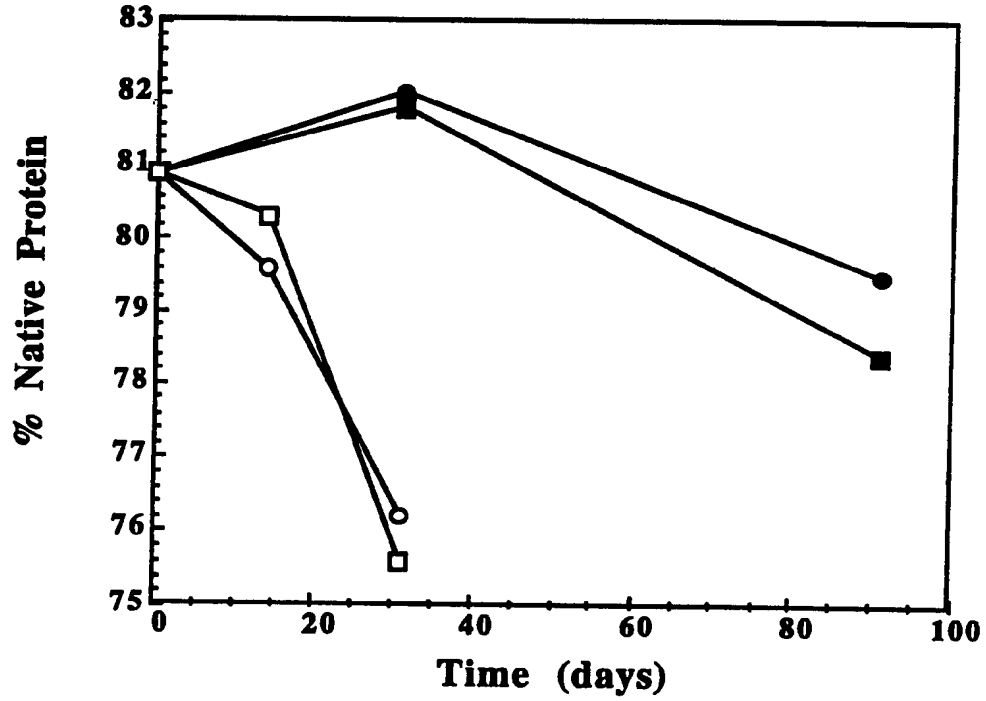


FIG. 7

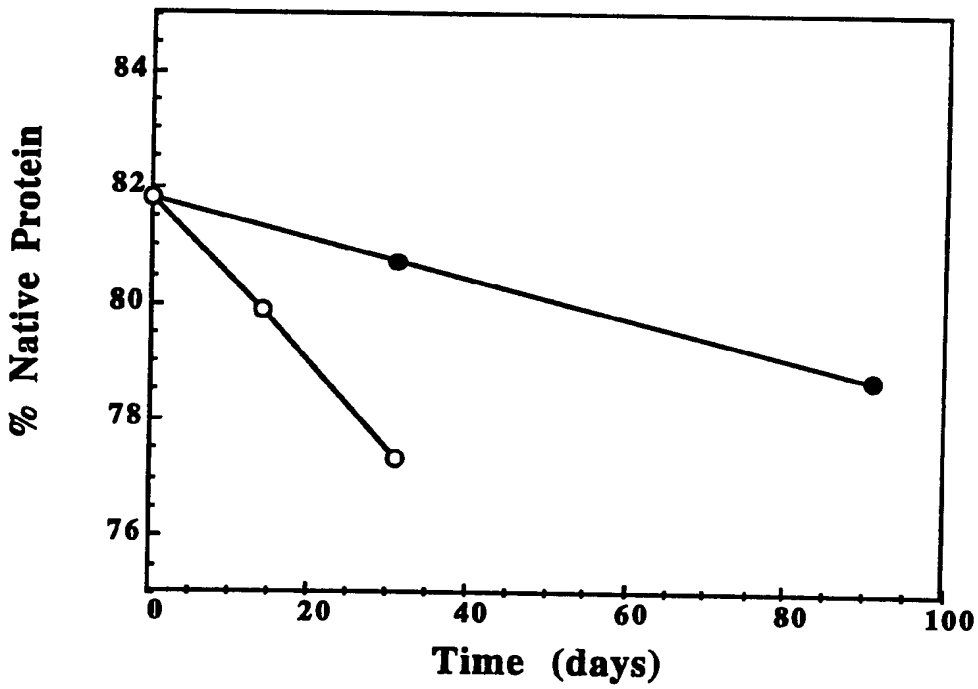


FIG. 8

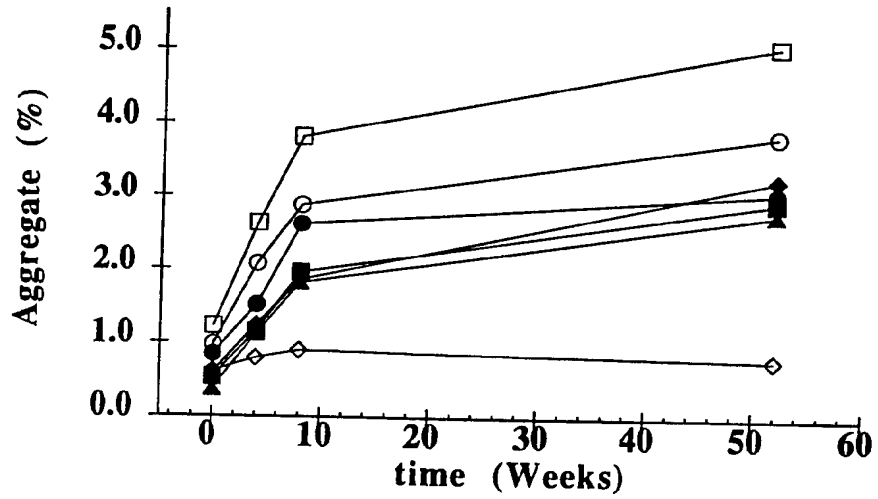


FIG. 9

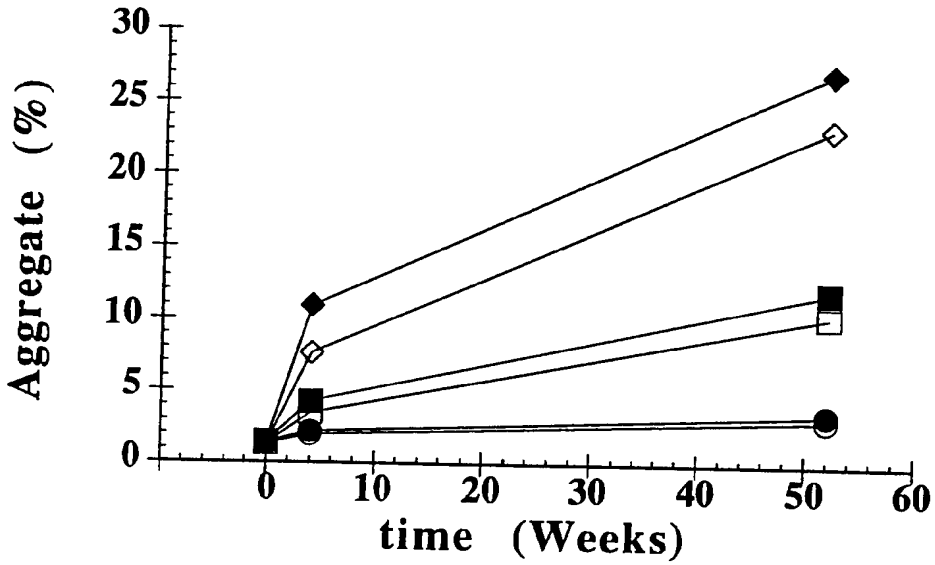


FIG. 10

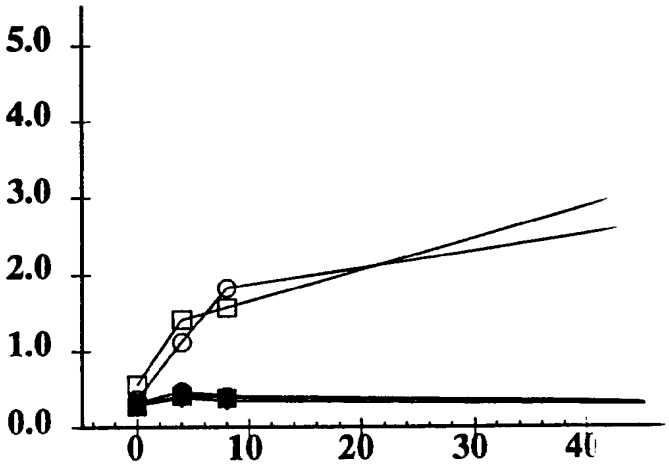


FIG. 11

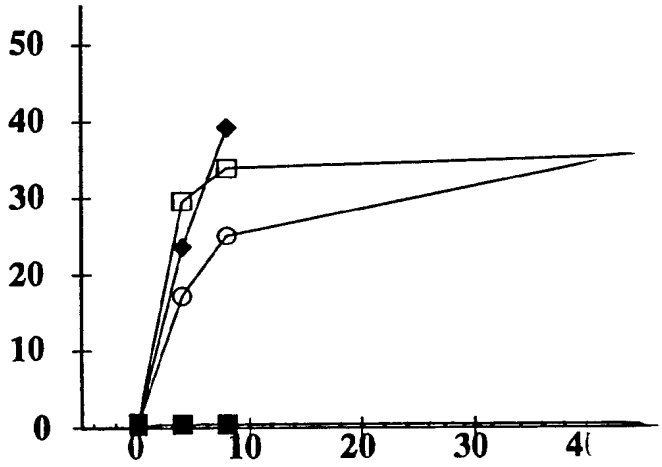


FIG. 12

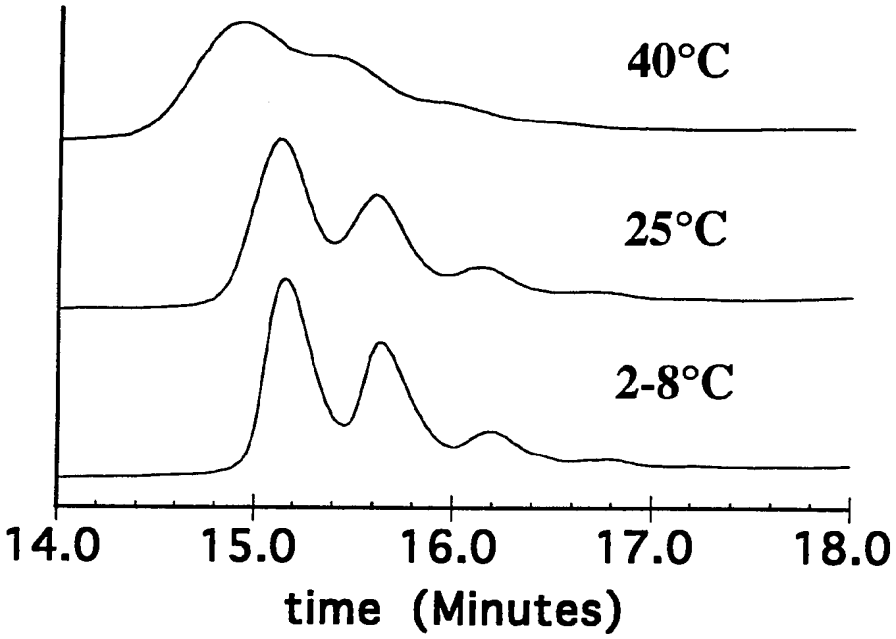


FIG. 13

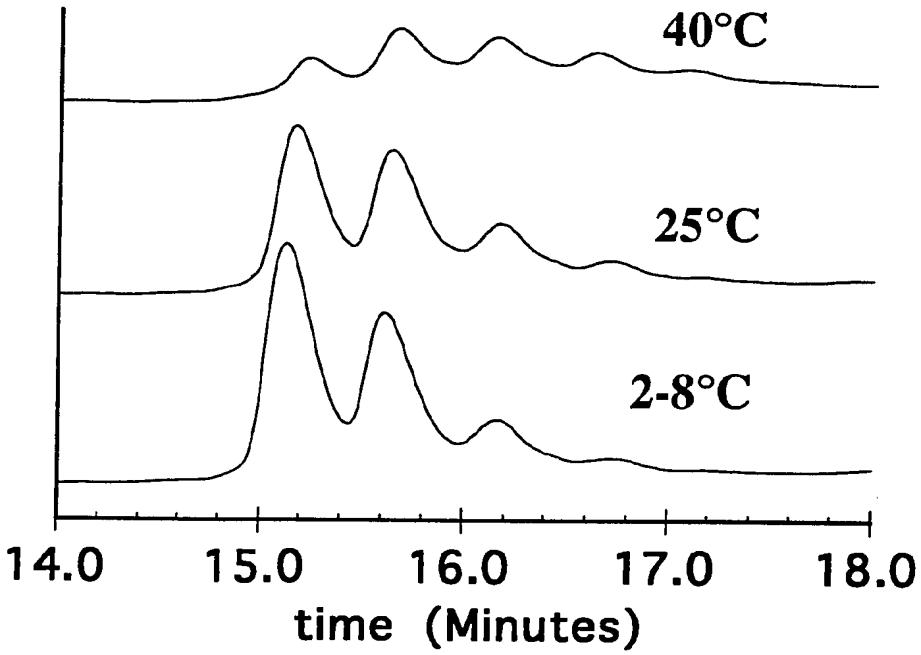


FIG. 14

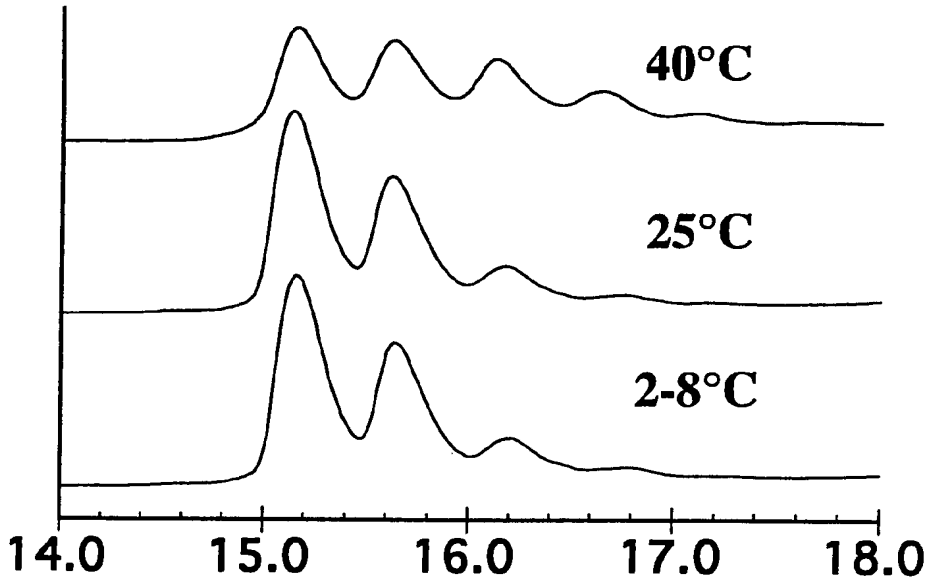


FIG. 15

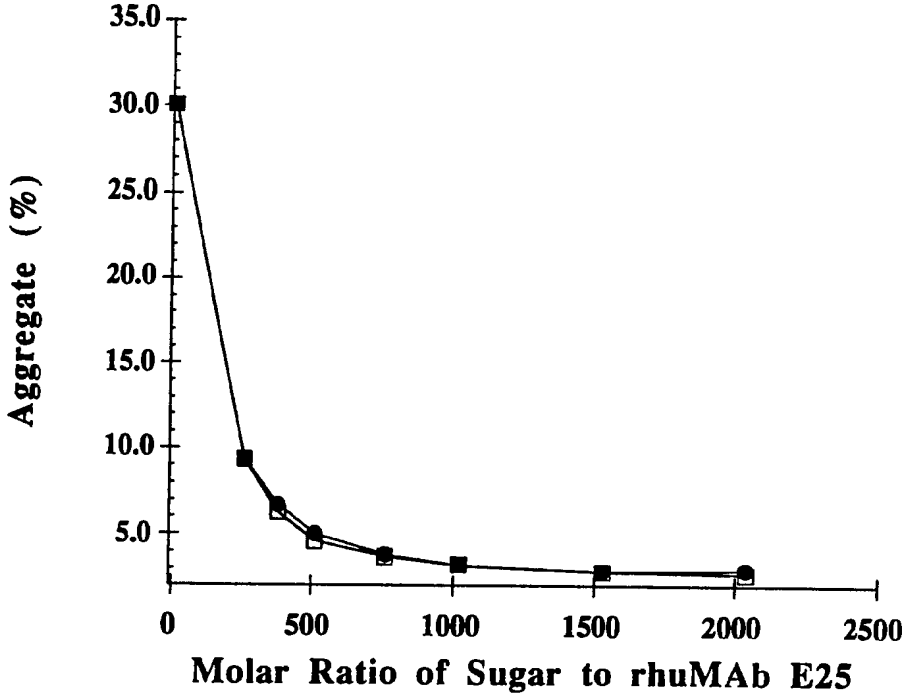


FIG. 16

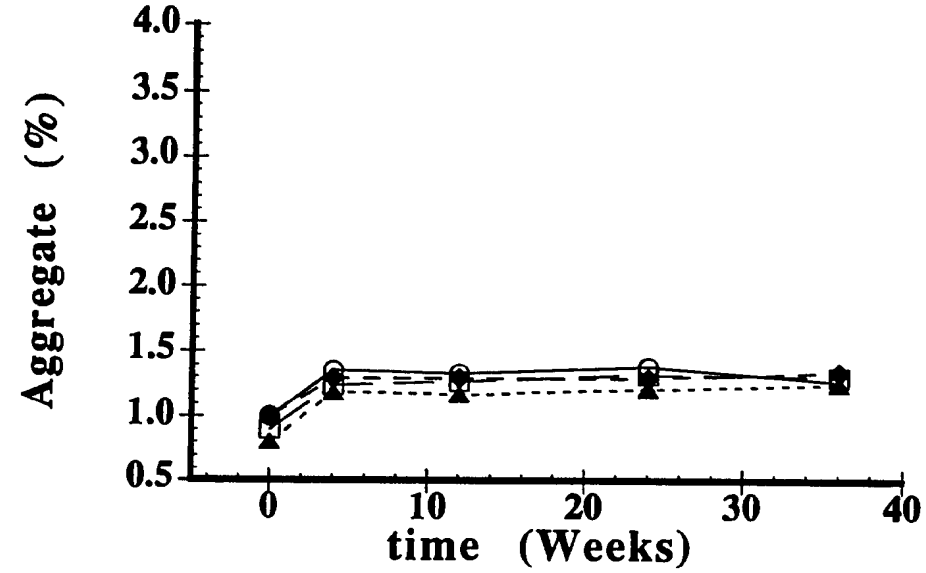


FIG. 17

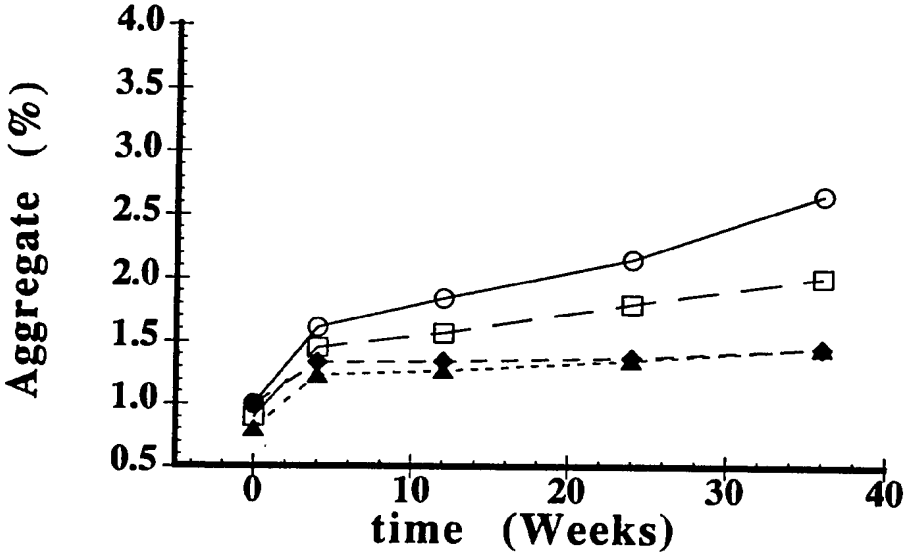


FIG. 18

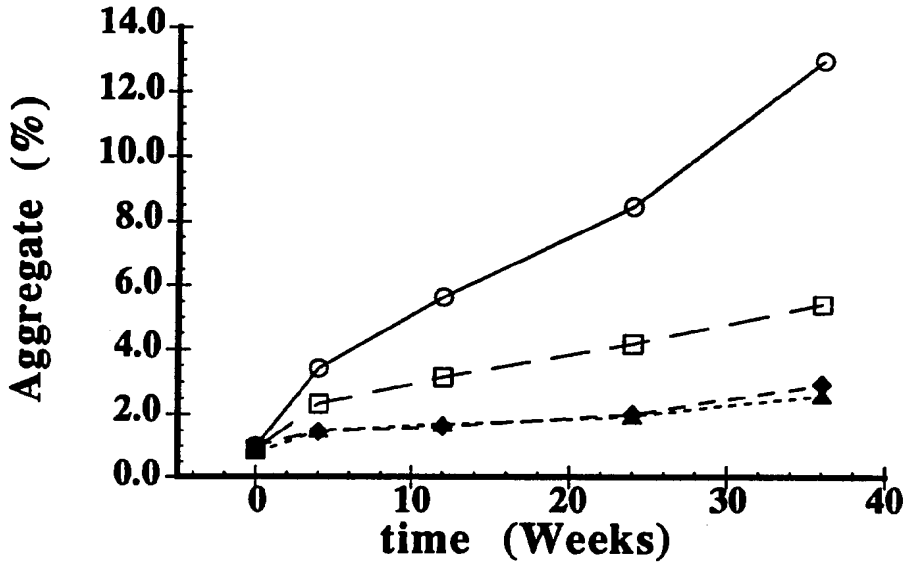


FIG. 19

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 96/12251

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/00 A61K39/395 C07K1/00

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 6 A61K C07K

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 661 060 A (IMMUNO AG) 5 July 1995 see the whole document ---	1,3,6, 8-12
A	WO 89 09402 A (TORAY INDUSTRIES INC.) 5 October 1989 see the whole document ---	1-3,6, 10,12
A	JOURNAL OF IMMUNOLOGICAL METHODS, vol. 181, no. 1, 12 April 1995, AMSTERDAM, NL, pages 37-43, XP002019425 P. DRABER ET AL.: "Stability of monoclonal IgM antibodies freeze-dried in the presence of trehalose." see abstract --- -/--	1-3,6, 10,12

[X] Further documents are listed in the continuation of box C.

[X] 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
25 November 1996	03.12.96

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	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF IMMUNOASSAY, vol. 16, no. 2, May 1995, NEW YORK, NY, USA, pages 183-197, XP000611616 K. NIELSEN ET AL.: "Stability of freeze-dried horseradish peroxidase-conjugated monoclonal antibodies used in diagnostic serology." see the whole document -----	1-3,6, 10,12

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/12251

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-661060	05-07-95	DE-C-	4344824	31-08-95
		CA-A-	2138853	29-06-95
		CZ-A-	9403284	12-07-95
		FI-A-	946104	29-06-95
		HU-A-	70449	30-10-95
		JP-A-	7206709	08-08-95
		NO-A-	945045	29-06-95
WO-A-8909402	05-10-89	AT-T-	117434	15-02-95
		DE-D-	68920693	02-03-95
		DE-T-	68920693	24-05-95
		EP-A-	0365685	02-05-90
		US-A-	5262296	16-11-93

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 2 663 325 A1

(12)

(43) Date of publication:
20.11.2013 Bulletin 2013/47

(21) Application number: 12700590.8

(22) Date of filing: **11.01.2012**

(51) Int. Cl.:
A61K 38/18 (2006.01) A61P 27/00 (2006.01)

(86) International application number:
PCT/US2012/020855

(87) International publication number:
WO 2012/097019 (19.07.2012 Gazette 2012/29)

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **13.01.2011 US 201161432245 P**
21.01.2011 US 201161434836 P
21.11.2011 US 201161561957 P

(71) Applicant: **Regeneron Pharmaceuticals, Inc.**
Tarrytown, NY 10591 (US)

(72) Inventor:
- **YANCOPOULOS, George D.**
Yorktown Heights NY 10598 (US)

(74) Representative: **Power, David**
J A Kemp 14 South Square Gray's Inn
London WC1R 5JJ
London WC1R 5JJ (GB)

(54) USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 July 2012 (19.07.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/097019 A1

(51) International Patent Classification:
A61K 38/18 (2006.01) *A61P 27/00* (2006.01)

(21) International Application Number:
PCT/US2012/020855

(22) International Filing Date:
11 January 2012 (11.01.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/432,245	13 January 2011 (13.01.2011)	US
61/434,836	21 January 2011 (21.01.2011)	US
61/561,957	21 November 2011 (21.11.2011)	US

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

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **YANCOPOULOS, George D.** [US/US]; 1519 Baptist Church Road, Yorktown Heights, NY 10598 (US).

(74) Agent: **COTTINGHAM, Frank**; Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, 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, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, 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, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

(57) 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 and corneal neovascularization.

WO 2012/097019 A1

USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

FIELD OF THE INVENTION

[0001] 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

[0002] 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.

[0003] 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.

[0004] 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

[0005] 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.).

[0006] 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.

[0007] 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."

[0008] 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).

[0009] 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.

[0010] Other embodiments of the present invention will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURE

[0011] 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

[0012] 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.

[0013] 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.).

[0014] 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

[0015] 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.

[0016] 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.

[0017] 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.

[0018] 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.

[0019] 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.

[0020] 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

[0021] 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.

[0022] 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").

[0023] 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.

[0024] 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

[0025] 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 choroidal neovascularization, age-related macular degeneration (AMD), diabetic retinopathies, diabetic macular edema (DME), central retinal vein occlusion (CRVO), corneal neovascularization, and retinal neovascularization.

PHARMACEUTICAL FORMULATIONS

[0026] 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.

[0027] 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

[0028] 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

[0029] 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.

[0030] 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.

[0031] 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

[0032] 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

[0033] 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.

[0034] 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

[0035] 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

[0036] 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

[0037] 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.

[0038] 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

[0039] 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.

[0040] 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.

[0041] 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.

[0042] 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

[0043] 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).

[0044] 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.

[0045] 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.

[0046] Approximately 1200 subjects were enrolled, with a target enrollment of 300 subjects per treatment arm.

[0047] 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.

[0048] 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.

[0049] 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).

[0050] 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.

[0051] 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.

[0052] The study procedures are summarized as follows:

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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.

[0057] 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.

[0058]

C. Results Summary (52 Week Data)

[0059] 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

[0060] 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.

[0061] 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)

[0062] 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 weeks[a] (2Q8)	42	8.5**	9.7**
VEGFT 2 mg as needed[a] (PRN)	45	10.3**	12.0**

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

[0063] 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

[0064] 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

[0065] 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.

[0066] 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.

[0067] 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.

SEQUENCES

[0068] SEQ ID NO:1 (DNA sequence having 1377 nucleotides):

ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGTCTGCTTCTC
ACAGGATCTAGTTCCGGAAGTGATACCGGTAGACCTTTCGTAGAGATGTACAGTGAAATCC
CCGAAATTATACACATGACTGAAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCAC
CTAACATCACTGTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGC
ATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGC
TTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAAACTATCTCACACATCGA
CAAACCAATACAATCATAGATGTGGTTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTGG
AGAAAAGCTTGTCTTAAATTGTACAGCAAGAACTGAACTAAATGTGGGGATTGACTTCAACT
GGGAATACCCTTCTTCGAAGCATCAGCATAAGAAACTTGTAAACCGAGACCTAAAAACCCA
GTCTGGGAGTGAGATGAAGAAATTTTTGAGCACCTTAACTATAGATGGTGTAACCCGGAGT
GACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACA
TTTGTCAGGGTCCATGAAAAGGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAA
CTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATC
TCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGT
CAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG
AGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT

GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC
CATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCT
ATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAG
ACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG
GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG
CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

[0069] SEQ ID NO:2 (polypeptide sequence having 458 amino acids):

MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITV
TLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLS
PSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLT
IDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

[0070] 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 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.

21. A VEGF antagonist for use in a method of treating an angiogenic eye disorder in a patient, wherein the method comprises 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.

22. The VEGF antagonist 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. The VEGF antagonist 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. The VEGF antagonist of any one of claims 21 to 23, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

25. The VEGF antagonist of any one of claims 21 to 23, 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. The VEGF antagonist of any one of claims 21 to 25, 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 and corneal neovascularization.

27. The VEGF antagonist of claim 26, wherein the angiogenic eye disorder is age related macular degeneration.

28. The VEGF antagonist of any one of claims 21 to 27, 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.

29. The VEGF antagonist of claim 28, wherein the VEGF antagonist is a VEGF receptor-based chimeric molecule.

30. The VEGF antagonist of claim 29, wherein the VEGF receptor-based chimeric molecule comprises VEGFR1R2-FcΔC1(a) encoded by the nucleic acid sequence of SEQ ID NO:1.

31. The VEGF antagonist of claim 29, 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.

32. The VEGF antagonist of any one of claims 21 to 31, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

33. The VEGF antagonist of claim 32, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

34. The VEGF antagonist of claim 33, wherein the intraocular administration is intravitreal administration.

35. The VEGF antagonist of claim 34, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

36. The VEGF antagonist of claim 35, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

37. The VEGF antagonist of claim 35, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

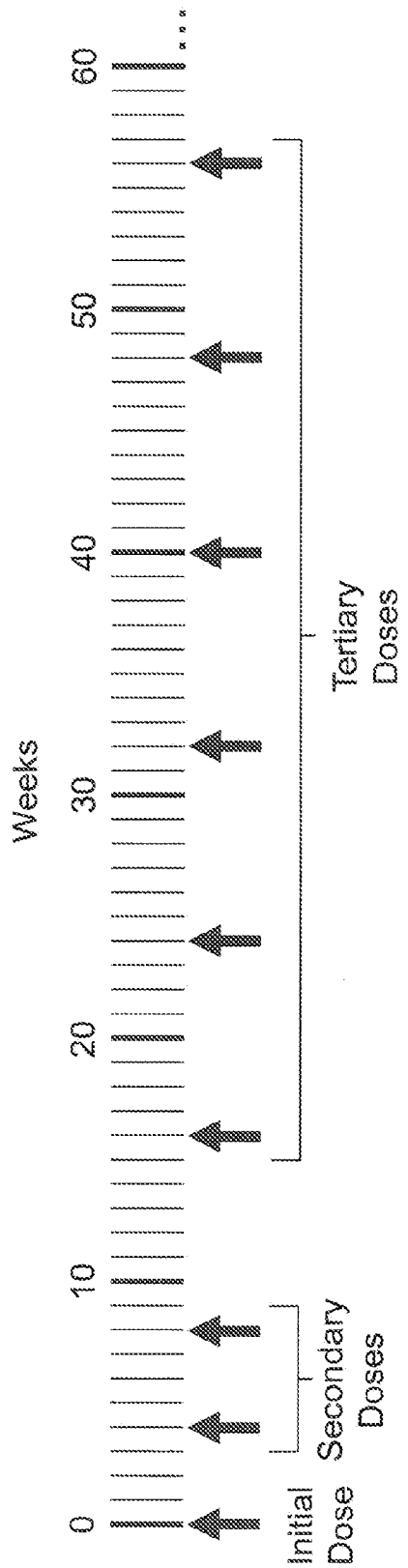

Weeks
0
10
20
30
40
50
60
...
Initial Dose
Secondary Doses
Tertiary Doses

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/020855

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/18 A61P27/00
ADD.

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 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 practicable, search terms used)
EPO-Internal, 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	Thomson Reuters Integrity: "VEGF Trap-Eye final phase II results in age-related macular degeneration presented at 2008 Retina Society Meeting", , 28 September 2008 (2008-09-28), pages 1-1, XP002674126, Retrieved from the Internet: URL:https://integrity.thomson-pharma.com/integrity/xmlxsl/pk_ref_list.xml_show_llist at_refs?p_session_id=1868065&p_orig=&p_count=354&p_num_dailys=42&p_qry_save=&p_subtitol=Biomedical%20Literature%20List&p_dailys=N&p_tsearch=A&p_text=N&p_whatToShow=REF&p_prousQuantity=5&p_page=10#link [retrieved on 2012-04-18] the whole document ----- -/--	1-37

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier application or patent 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
19 April 2012	22/05/2012

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, Fax: (+31-70) 340-3016	Rodrigo-Simón, Ana

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NGUYEN Q D ET AL: "A Phase I Study of Intravitreal Vascular Endothelial Growth Factor Trap-Eye in Patients with Neovascular Age-Related Macular Degeneration", OPHTHALMOLOGY, J. B. LIPPINCOTT CO., PHILADELPHIA, PA, US, vol. 116, no. 11, 1 November 2009 (2009-11-01), pages 2141-2148.E1, XP026732998, ISSN: 0161-6420, DOI: 10.1016/J.OPHTHA.2009.04.030 [retrieved on 2009-08-22] abstract page 2142, column 1 - paragraph 3 page 2147, column 1, paragraph 2	1-37
A	US 2007/190058 A1 (SHAMS NAVEED [US]) 16 August 2007 (2007-08-16) claim 1 paragraph [0112] page 12, paragraph 111	1-37
A	US 2006/172944 A1 (WIEGAND STANLEY J [US] ET AL) 3 August 2006 (2006-08-03) paragraphs [0008], [0017] claims 1,2	1-37
A	US 2005/163798 A1 (PAPADOPOULOS NICHOLAS J [US] ET AL) 28 July 2005 (2005-07-28) claim 65 paragraph [0122]	1-37
A	WO 00/75319 A1 (REGENERON PHARMA [US]; PAPADOPOULOS NICHOLAS J [US]; DAVIS SAMUEL [US]) 14 December 2000 (2000-12-14) page 87, line 14 - line 88 page 14, line 7 - line 12	1-37
A	US 2006/058234 A1 (DALY THOMAS J [US] ET AL) 16 March 2006 (2006-03-16) paragraph [0067] claim 23 page 2, paragraph 15	1-37
A	US 2005/260203 A1 (WIEGAND STANLEY J [US] ET AL) 24 November 2005 (2005-11-24) page 7; example 8 page 8; example 9 page 8; example 11 page 9; example 2	1-37

-/--

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DO DV 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 OPHTHALMOL, vol. 93, no. 2, February 2009 (2009-02), pages 144-1449, XP009158490, abstract page 149, paragraph 2	1-37
A	ANANT PAI ET AL.: "Current concepts in intravitreal drug therapy for diabetic retinopathy", SAUDI JOURNAL OF OPHTHALMOLOGY, vol. 24, no. 4, 30 June 2010 (2010-06-30), pages 143-149, XP002674122, page 147, paragraph 4	1-37
X,P	DO DIANA V ET AL: "The DA VINCI Study: phase 2 primary results of VEGF Trap-Eye in patients with diabetic macular edema.", OPHTHALMOLOGY SEP 2011 LNKD-PUBMED:21546089, vol. 118, no. 9, September 2011 (2011-09), pages 1819-1826, XP002674123, ISSN: 1549-4713 the whole document	1-37
T	STEWART MW.: "The expanding role of vascular endothelial growth factor inhibitors in ophthalmology", MAYO CLIN PROC, vol. 87, no. 1, January 2012 (2012-01), pages 77-88, XP002674124, the whole document	
A	anonymous: "LUCENTIS® (ranibizumab injection) Intravitreal Injection", , June 2006 (2006-06), pages 1-3, XP002674125, Retrieved from the Internet: URL:http://www.gene.com/gene/products/information/pdf/lucentis-prescribing.pdf [retrieved on 2012-04-18] page 1, column 1, line 20 - page 1, column 2, line 9	1-37

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/020855

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2007190058 A1	16-08-2007	NONE	
US 2006172944 A1	03-08-2006	US 2006172944 A1	03-08-2006
		US 2008085276 A1	10-04-2008
US 2005163798 A1	28-07-2005	US 7070959 B1	04-07-2006
		US 2005163798 A1	28-07-2005
		US 2005175610 A1	11-08-2005
		US 2005245447 A1	03-11-2005
		US 2008194460 A1	14-08-2008
		US 2008194799 A1	14-08-2008
		US 2008220004 A1	11-09-2008
		US 2009081217 A1	26-03-2009
		US 2009155899 A1	18-06-2009
		US 2010221782 A1	02-09-2010
		US 2011028698 A1	03-02-2011
		US 2012064621 A1	15-03-2012
WO 0075319 A1	14-12-2000	AT 293164 T	15-04-2005
		AT 417928 T	15-01-2009
		AU 779303 B2	13-01-2005
		AU 5040400 A	28-12-2000
		BR 0011407 A	02-04-2002
		CA 2376379 A1	14-12-2000
		CN 1369009 A	11-09-2002
		CN 101433715 A	20-05-2009
		CZ 20014387 A3	16-10-2002
		DE 60019415 D1	19-05-2005
		DE 60019415 T2	09-03-2006
		DK 1544299 T3	23-03-2009
		EP 1183353 A1	06-03-2002
		EP 1544299 A1	22-06-2005
		ES 2237429 T3	01-08-2005
		ES 2319305 T3	06-05-2009
		HK 1043388 A1	10-06-2005
		HR 20010908 A2	30-04-2005
		HU 0201515 A2	28-08-2002
		IL 146890 A	08-07-2008
		JP 4723140 B2	13-07-2011
		JP 2003501089 A	14-01-2003
		JP 2011024595 A	10-02-2011
		MX PA01012630 A	22-07-2002
		NO 20016036 A	08-02-2002
		NZ 515913 A	30-01-2004
		PL 352246 A1	11-08-2003
		PT 1183353 E	30-06-2005
		PT 1544299 E	18-03-2009
		SK 17522001 A3	01-04-2003
		WO 0075319 A1	14-12-2000
		ZA 200110068 A	06-12-2002
US 2006058234 A1	16-03-2006	NONE	
US 2005260203 A1	24-11-2005	NONE	

Electronic Acknowledgement Receipt

EFS ID:	43190093
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	08-JUL-2021
Filing Date:	16-OCT-2020
Time Stamp:	20:21:32
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-008CIPCON6_2021-07-08_SuppIDS_Trans.pdf	52509 9396af8dad2ef9d5bbf8a4b1406d91c66a672d1f	no	3

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	Substitute_1449_2021-07-08_17072417_REGN-008CIPCON6.pdf	78570 b294d2d34e50470c3cd6cab84619c67c8a95c4a4	no	8
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Foreign Reference	WO1997004801A1.pdf	2385872 d6c7f09de2b293ea071cd375ab1254ba1983c6ca	no	49
Warnings:					
Information:					
4	Foreign Reference	EP_2663325A1.pdf	3386809 9843d249b68b7d09ed3ae87ba681084271f56478	no	29
Warnings:					
Information:					
5	Non Patent Literature	Adis_2008-IPR2021_00880_Exhibit_1007.pdf	645161 8ceeb6f0a03e9ab6f37944e84bc938e5d40b11a3	no	10
Warnings:					
Information:					
6	Non Patent Literature	Andersen_and_Krummen_2002.pdf	518664 358a53f9fbe31a1c749c4c9b000423fd807118a6	no	7
Warnings:					
Information:					
7	Non Patent Literature	Anderson_2010.pdf	6117589 2af7b6b68a9dc8b746f2027ea8abba17f5247b82	no	13
Warnings:					
Information:					
8	Non Patent Literature	Article_in_Retinal_Physician_Subspecialty_News_March_2010.pdf	798263 331dea26ccde9f5bfa737258bb734796777d7e9d	no	4
Warnings:					
Information:					

9	Non Patent Literature	Assn_for_Res_Vision_Opthalmology_ARVO_New_Winter_Spring_2008.pdf	2200202 774f0d9debf9cfe72073f30977ba98d242d064db	no	20
Warnings:					
Information:					
10	Non Patent Literature	Assn_for_Res_Vision_Opthalmology_ARVO_News_Summer_2007.pdf	2708366 53999edd2f47e4db6cb24b29a0064f07a1a60f07	no	28
Warnings:					
Information:					
11	Non Patent Literature	AVASTIN_label.pdf	1148926 5223ff5f93c1d34ec290bcb03bd7e352ef6f65f4	no	37
Warnings:					
Information:					
12	Non Patent Literature	Avery_2006.pdf	8344801 76b32182f8d41a8f51c7343fec002dc4886952f1	no	15
Warnings:					
Information:					
13	Non Patent Literature	Bashshur_2006.pdf	3655748 c7bcdb6adc0b06aee24fc619c92811a5f10a4eb4	no	9
Warnings:					
Information:					
14	Non Patent Literature	Bayer_Press_Release_27_February_18_2010.pdf	228698 be55660194592aa91a9a9117a8f2307d76884a9a	no	8
Warnings:					
Information:					
15	Non Patent Literature	Bayer_Press_Release_28_April_28_2008.pdf	199778 9738af76594d104a414816c2224e1432a9f5cdb1	no	7
Warnings:					
Information:					

16	Non Patent Literature	Bayer_Press_Release_29_August_19_2008.pdf	188772 24a3d04f50fdeab1678afd6dc6caeb76553112f5	no	6
Warnings:					
Information:					
17	Non Patent Literature	Bayer_Regeneron_3_Study_for_VEGF_Trap-Eye_May_8_2008.pdf	97152 55f8a0232fb37576f3d7edab1ffffc392dbc05d2	no	2
Warnings:					
Information:					
18	Non Patent Literature	BMJ_Publishing_Group_Ltd.pdf	365125 63ec18c39d4b83ca2537c1d407ba784e08cb5b48	no	12
Warnings:					
Information:					
19	Non Patent Literature	Bressler_2001.pdf	6309600 d2e7dd7352a6bc52309499e3922eee0774a49d56	no	10
Warnings:					
Information:					
20	Non Patent Literature	Brown_and_Regillo_2007.pdf	4162692 c6ff93af1f52ac4b12036a96d9728690c73d4b40	no	13
Warnings:					
Information:					
21	Non Patent Literature	Chi_September_2003.pdf	216608 2e0af95eb7e18d87e70a816c4fcc57bd5a20149a	no	12
Warnings:					
Information:					
22	Non Patent Literature	Ciulla_and_Rosenfeld_2009.pdf	151148 3fbdcc89116daa9234ba1f73ec336636f1447dc6	no	8
Warnings:					
Information:					

23	Non Patent Literature	ClinicalTrials_gov_36_VEGF_Trap-Eye_August_2008.pdf	243373 3da614c521013ec8ab75095a9007de42ef1b17a3	no	27
Warnings:					
Information:					
24	Non Patent Literature	Clinicaltrials_gov_37_1-SPV-2_Trial_2010.pdf	282705 3f0b068e3dd3ff498b59259890abb8ae07d9f04c	no	13
Warnings:					
Information:					
25	Non Patent Literature	Clinicaltrials_gov_38_Double-Masked_Study_of_Efficacy_June_2008.pdf	211779 2a2e3321a04e9b257e1d5ab085edeba72f430d22	no	23
Warnings:					
Information:					
26	Non Patent Literature	CMS_Local_Coverage_Determination_June_14_2011.pdf	248026 00f31803334917db10895d203b413b2648dfd422	no	4
Warnings:					
Information:					
27	Non Patent Literature	Controls_in_SCI_experiments_RegenBase.pdf	352383 46f38c72105ece7c4c1d6fc3c1238185810be43f	no	2
Warnings:					
Information:					
28	Non Patent Literature	Department_of_Health_and_Human_Services_September_2015.pdf	8547221 4fb3406eac10cfb893beb8ec79e775f52ffd8d6d	no	35
Warnings:					
Information:					
29	Non Patent Literature	Drug_Vehicle_Code_C927_National_Cancer_Institute_NCI_retrieved_January_6_2021.pdf	445390 348d453268aa86038e59fa063c12efa8f3717010	no	1
Warnings:					
Information:					

30	Non Patent Literature	Eylea_Prescribing_Information_Revised_05-2019.pdf	1044796 8db25ef1770d98cce33d190e64881c2a717c7c43	no	29
Warnings:					
Information:					
31	Non Patent Literature	Ferrara_2005.pdf	489150 646845e11460e7105473dace9952ee42ba516de1	no	8
Warnings:					
Information:					
32	Non Patent Literature	Fraser_2005.pdf	730741 736efdc2ab9d7cacc96df2be584549ce26f99d69	no	9
Warnings:					
Information:					
33	Non Patent Literature	Genentech_FDA_Approves_Lucentis_Release_dated_June_30_2006.pdf	860707 97fab5f31ed80176a2491e4fc171aff3456c8fbe	no	6
Warnings:					
Information:					
34	Non Patent Literature	Gupta_2010.pdf	1938717 e7427d381b85ee548600ddd84c7ddadb0a69109e	no	7
Warnings:					
Information:					
35	Non Patent Literature	Heier_2012.pdf	4280253 3f4d3291331e74184875df0adc6a95541f142dd1	no	8
Warnings:					
Information:					
36	Non Patent Literature	Heier_Intravetrial_VEGF_Trap_October_2009.pdf	136012 a75ad81cfba173db31252b0685238853091 46da1	no	2
Warnings:					
Information:					

37	Non Patent Literature	HERCEPTIN_label.pdf	198202 6801eb4aeb9468b1c4352520f1961687a7b69925	no	2
Warnings:					
Information:					
38	Non Patent Literature	Holz_2013.pdf	400441 ddd7ab20049cc400ebfed823e2921b053377f6a6	no	7
Warnings:					
Information:					
39	Non Patent Literature	Ip_2009.pdf	16234645 4631f24f147f31fe63874140a029ad7064061da4	no	16
Warnings:					
Information:					
40	Non Patent Literature	Janeway_2001.pdf	233188 a2ff06b0a12587a28742c945decbaa4a5cef7f27	no	6
Warnings:					
Information:					
41	Non Patent Literature	Keane_2009.pdf	834744 05c5db4d5384c621839d922675dddee8f5405472	no	9
Warnings:					
Information:					
42	Non Patent Literature	Kim_2002.pdf	1521858 5bd8f58481390f3458b348df9636a7ebc77e8242	no	6
Warnings:					
Information:					
43	Non Patent Literature	LUCENTIS_Label.pdf	333618 8d1edaf651437070430 4da849c2fe3214cc4a067	no	14
Warnings:					
Information:					

44	Non Patent Literature	LUCENTIS_Prescribing_Information_2006.pdf	284762 afa80c932c89d06fdf8c906a1586e7d5c8c2c641	no	7
Warnings:					
Information:					
45	Non Patent Literature	Macular_Photocoagulation_Study_G_1991.pdf	7281266 b73e1aa37a17fbd4d2d1fd412bac0ab9404b4b96	no	12
Warnings:					
Information:					
46	Non Patent Literature	Massin_Sept_Oct_2008.pdf	113452 4cca933d0cd781843cf7426f05ee65b118b00d5b	no	3
Warnings:					
Information:					
47	Non Patent Literature	Michels_2005.pdf	8279802 054d1627da4550590599763b7dc2525cc73cea61	no	22
Warnings:					
Information:					
48	Non Patent Literature	Ni_and_Hui_2009.pdf	757983 6ef02b7b8eb70cffc8666caceb715b2513d5d9a9	no	12
Warnings:					
Information:					
49	Non Patent Literature	Parkins_and_Lashmar_April_4_2000.pdf	8867718 a0b9644ecb2b5fc211569573ab5017214d18cba0	no	9
Warnings:					
Information:					
50	Non Patent Literature	Phosphate_buffer_Cold_Spring_Harbor_Protocols_2006.pdf	48874 b886c50d4d1c6b60bfc3ef106080b60f1b2915a4	no	1
Warnings:					
Information:					

51	Non Patent Literature	Randolph_and_Jones_2002.pdf	1723719 724131c7ed3f72517bd126616c16643ce2317727	no	17
Warnings:					
Information:					
52	Non Patent Literature	RAPTIVA_label.pdf	505726 4a1f99c2d9b9ff08441f6e2093e55e639a37ba73	no	36
Warnings:					
Information:					
53	Non Patent Literature	Regeneron_Receives_20_Million_Milestone_14_Aug_2007.pdf	42708 19c5fe875d48483fda7833125485ab89f68f88d2	no	2
Warnings:					
Information:					
54	Non Patent Literature	Regeneron_Reports_Fourth_Quarter_22_feb_2005.pdf	113291 b381ff9dd041f8403d7b4593db967848407a005f	no	8
Warnings:					
Information:					
55	Non Patent Literature	Regeneron_Reports_Fourth_Quarter_24_Feb_2006.pdf	131395 1667fbbd157a406d44edeb9101bf12c921abaff1	no	13
Warnings:					
Information:					
56	Non Patent Literature	Regeneron_Reports_Positive_Phase_1_Data_1_May_2006).pdf	68846 27d9139931950daf5af1431af387a5d6771ba8a6	no	4
Warnings:					
Information:					
		Total Files Size (in bytes):	111748544		

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	Attorney Docket No.	REGN-008CIPCON6
	Confirmation No.	7325
	First Named Inventor	George D. Yancopoulos
	Application Number	17/072,417
	Filing Date	October 16, 2020
	Group Art Unit	
Address to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Examiner Name	
	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

Applicant submits 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 publications discussed herein are provided to comply with the duty to disclose in accordance with 37 C.F.R. § 1.56. However, nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

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 documents 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-008CIPCON6.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 8 July 2021

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
Reg. No. 28,807

BOZICEVIC, FIELD & FRANCIS LLP
201 Redwood Shores Parkway, Suite 200
Redwood City, CA 94065
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

Electronic Acknowledgement Receipt

EFS ID:	43204568
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	08-JUL-2021
Filing Date:	16-OCT-2020
Time Stamp:	20:22:32
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	Non Patent Literature	7374758_PTEAppln_2011-12-22_pt1.pdf	5809055 0640265a90881d7c41e847aed51db7a77d55a507	no	50

Warnings:

Information:					
2	Non Patent Literature	7374758_PTEAppln_2011-12-22_pt2.pdf	5343343 95b18f744daa7632161ff99b6584230b30cabb82	no	50
Warnings:					
Information:					
3	Non Patent Literature	7374758_PTEAppln_2011-12-22_pt3.pdf	7962144 731a22c71cbec2c8e90df22ffbe1bfd150f1d5c4	no	50
Warnings:					
Information:					
4	Non Patent Literature	7374758_PTEAppln_2011-12-22_pt4.pdf	11609355 9c69ae1e6dfbb292c744bf9ab295a1139ea5d27b	no	48
Warnings:					
Information:					
5	Non Patent Literature	EP2663325_File_History_Pt1.pdf	20194681 d2967a667e9dd08e1a70cd56ba2a0fac1c40211f	no	150
Warnings:					
Information:					
6	Non Patent Literature	EP2663325_File_History_Pt2.pdf	24931781 350d5ff0fbda840bb21a1204ae586ccb68d0876d	no	150
Warnings:					
Information:					
7	Non Patent Literature	EP2663325_File_History_Pt3.pdf	16757198 c4a043244cd800a6a6ebccd6bf64be3392d88aec	no	150
Warnings:					
Information:					
8	Non Patent Literature	EP2663325_File_History_Pt4.pdf	12749186 9edea07222761eeda868e1b50231a634653a8ef3	no	75
Warnings:					
Information:					

9	Non Patent Literature	EP2663325_File_History_Pt4A.pdf	15286735 184cec1d59f9b7354931255bd961d26877a4ef1d	no	75
Warnings:					
Information:					
10	Non Patent Literature	EP2663325_File_History_Pt5.pdf	7244079 7f625a8e968015fbb52a2d94fa20d0bb5985cb10	no	89
Warnings:					
Information:					
11	Non Patent Literature	Mitchell_2009_sm.pdf	9041481 bae0fa36240ddb4a9774e10b8fec412cfd84881a	no	15
Warnings:					
Information:					
12	Non Patent Literature	Regeneron_Reports_Positive_Phase_1_Data_1_May_2006.pdf	68846 27d9139931950daf5af1431af387a5d6771ba8a6	no	4
Warnings:					
Information:					
13	Non Patent Literature	Regeneron_SEC_Form_10-Q_September_30_2009.pdf	520308 45e7f23b9091ede2efca7087200c36ed5b588f6f	no	55
Warnings:					
Information:					
14	Non Patent Literature	Reichert_2011_Exhibit_1072.pdf	812474 568856b847cd3e244db0bc5a219a7e7644e9af21	no	25
Warnings:					
Information:					
15	Non Patent Literature	REMICADE_label.pdf	1775753 f20e055f4abcfae3478fc4d00e4cfcaf34e23160	no	58
Warnings:					
Information:					

16	Non Patent Literature	Retina_Coding_QA_Retinal_Physician_July-August_2019.pdf	164558 c03344a2b83fcf679d2bcbf2c60648533f8c2979	no	4
Warnings:					
Information:					
17	Non Patent Literature	Rogers_2010.pdf	1901724 0b7c5678713078077ea535e3a285e58cf0c9dcbe	no	8
Warnings:					
Information:					
18	Non Patent Literature	Rosenfeld_2005.pdf	8265168 bf4201caecc9e7a978b6a0a0d4094ec08e09e36a	no	5
Warnings:					
Information:					
19	Non Patent Literature	Rudge_2005.pdf	179808 6e7b500b335f5da39c607088a03d60f8ba93b453	no	8
Warnings:					
Information:					
20	Non Patent Literature	Schmidt-Erfurth_2010.pdf	192176 7a078ab58c0545c91bd0aecbe36ba739ec582570	no	8
Warnings:					
Information:					
21	Non Patent Literature	Scott_2009.pdf	14428548 a3f130fad3a9028112184fb8c5d045b7b63ffb3f	no	15
Warnings:					
Information:					
22	Non Patent Literature	SIMULECT_label.pdf	880267 ce5e99b9b6463dcaafb893c876b9777301518dba	no	7
Warnings:					
Information:					

23	Non Patent Literature	Spaide_2009.pdf	3968288 5a8e1fedbdbb4d0c3d4ae3d77f8b6f5e94a516c8	no	9
Warnings:					
Information:					
24	Non Patent Literature	Spielberg_2009.pdf	593596 8b82730715af713b00463b1cf055c55a9a09695a	no	12
Warnings:					
Information:					
25	Non Patent Literature	Steinbrook_2006.pdf	704385 366b389685c3448b71c64fa0c6c9f02590068017	no	4
Warnings:					
Information:					
26	Non Patent Literature	The_Branch_Vein_Occlusion_Study_G_Am_J_Opthal_1984.pdf	2329843 c3627454a192941d670d195319b54feda98e35bf	no	12
Warnings:					
Information:					
27	Non Patent Literature	The_Central_Vein_Occlusion_Study_G_Ophthal_1995.pdf	7219556 bdceb3fe123c6677908a992623547acc35b3a606	no	9
Warnings:					
Information:					
28	Non Patent Literature	US_DEPT_HEALTH_HUMAN_SERVS_87_NATL_INST_HEALTH_Sept_2015.pdf	1056059 01b7e427ffff08cd7a3d2cbfa845624df25570ca	no	32
Warnings:					
Information:					
29	Non Patent Literature	US_DEPT_HEALTH_HUMAN_SERVS_88_NATL_INST_HEALTH_Sept_2015.pdf	3957066 64dd7b0c500bc4b6c899682f9b1a2296f26d2ec5	no	20
Warnings:					
Information:					

30	Non Patent Literature	US_DEPT_HEALTH_HUMAN_SERVS_89_Nov_2003.pdf	1578429 fb52d7653abe99e01b043788f4848ba492f0841a	no	25
Warnings:					
Information:					
31	Non Patent Literature	VEGF_Efficacy_Safety_2009-04-28.pdf	281732 46cb2b447eaa3e82bdf10ac26a3eec9c0acb16f7	no	27
Warnings:					
Information:					
32	Non Patent Literature	Wall_Street_Journal_2007.pdf	165209 809fec1f231f793922d8d9794b78c7fe0c62f75e	no	5
Warnings:					
Information:					
33	Non Patent Literature	Wulf_July_2002.pdf	2632226 3a46bc4f9056a1a7cf3ea2568441b0ffe1d24e50	no	11
Warnings:					
Information:					
34	Non Patent Literature	XOLAIR_label.pdf	230047 41ecd7656832789c122ad7cad7da0e452a6f9aa8	no	17
Warnings:					
Information:					
35	Non Patent Literature	Zarbin_and_Rosenfeld_2010.pdf	3104891 5f4e668b1b9b4c4ebdffb9c6f2d3059da9d981c4	no	20
Warnings:					
Information:					
			Total Files Size (in bytes):	193939995	

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.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	17/072,417
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	1	of	2	Attorney Docket Number	REGN-008CIPCON6

U.S. PATENT DOCUMENTS					
Examiner Initial*	Cite No.	Patent Number Number-Kind Code (*if known*)	Issue Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1				

U.S. PATENT APPLICATION PUBLICATIONS					
Examiner Initial*	Cite No.	Publication Number **Number-Kind Code** (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1				

FOREIGN PATENT DOCUMENTS						
Examiner Initial*	Cite No.	Foreign Document Number Country Code-Number-Kind Code (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	1					

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	1	Eylea®, Highlights of Prescribing Information, Revised 11/2011		
	2	IPR2021-00880, Paper 1, Petition for IPR (May 5, 2021)		
	3	IPR2021-00880, Exhibit 1002, Albini Declaration (May 4, 2021)		
	4	IPR2021-00880, Exhibit 1003, Gerritsen Declaration (April 30, 2021)		
	5	IPR2021-00880, Paper 10, Preliminary Response of Patent Owner (August 16, 2021)		
	6	IPR2021-00881, Paper 1, Petition for IPR (May 5, 2021)		
	7	IPR2021-00881, Exhibit 1002, Albini Declaration (May 4, 2021)		
	8	IPR2021-00881, Exhibit 1003, Gerritsen Declaration (April 26, 2021)		
	9	IPR2021-00881, Paper 10, Preliminary Response of Patent Owner (August 16, 2021)		
	10	IPR2021-00881, Exhibit 2001, Do Declaration (August 13, 2021)		
	11	Mitchell *et al.*, "Evaluating the Impact of Intravitreal Aflibercept on Diabetic Retinopathy Progression in the VIVID-DME and VISTA-DME Studies" Ophthalmol Retina 2(10):988-96 (2018)		
	12	PGR2021-00035, Paper 2, Petition for PGR (January 7, 2021)		
	13	PGR2021-00035, Paper 6, Preliminary Response of Patent Owner (April 15, 2021)		
	14	PGR2021-00035, Exhibit 1003 Wu Declaration (January 7, 2021)		
	15	PGR2021-00035, Exhibit 2001 Do Declaration (April 14, 2021)		
	16	PGR2021-00035, Exhibit 2002 D. Brown Declaration (April 14, 2021)		
	17	CAO, J. R., R.; Wang, Q.; Yancopoulos, G.D.; Wiegand, S.J. (2002). Inhibition of Corneal Neovascularization and Inflammation by VEGF Trap. In "ARVO", Invest. Ophthalmol. Vis. Sci. Vol. 43. E-Abstract 1863		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	17/072,417
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	2	of	2	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	18	WANG, Q. R., R.; Cao, J.; Yancopoulos, G.D.; and Wiegand, S.J. (2002). Anti-Angiogenic Properties of a New VEGF Antagonist, VEGF Trap, in a Mouse Model of Retinal Neovascularization. In "ARVO", Invest. Ophthalmol. Vis. Sci., Vol. 43. E-Abstract. 3714		
	19	SAISHIN, Y., Saishin, Y., Takahashi, K., Lima e Silva, R., *et al.* (2003). VEGF-TRAP(R1R2) suppresses choroidal neovascularization and VEGF-induced breakdown of the blood-retinal barrier. J Cell Physiol 195:241-48		
	20	CURSIEFEN, C., Cao, J., Chen, L., Liu, Y., Maruyama, K., *et al.* (2004). Inhibition of hemangiogenesis and lymphangiogenesis after normal-risk corneal transplantation by neutralizing VEGF promotes graft survival. Invest Ophthalmol Vis Sci 45(8):2666-73		
	21	CURSIEFEN, C., Chen, L., Borges, L. P., Jackson, D., Cao, J., *et al.* (2004). VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. J Clin Invest 113(7):1040-50		
	22	CAO, J.; Song, H.; Renard, R.A.; Liu, Y.; Yancopolous, G.D.; Wiegand, S.J. (2005). Systemic Administration of VEGF Trap Suppresses Vascular Leak and Leukostasis in the Retinas of Diabetic Rats. In "ARVO", Vol. 46. Invest. Ophthalmol. Vis. Sci. E-Abstract 446		
	23	NORK, T. M., Dubielzig, R. R., Christian, B. J., Miller, P. E., Miller, J. M., *et al.* (2011). Prevention of experimental choroidal neovascularization and resolution of active lesions by VEGF trap in nonhuman primates. Arch Ophthalmol 129(8):1042-52		

Examiner Signature		Date Considered	

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

Electronic Acknowledgement Receipt

EFS ID:	43676685
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	03-SEP-2021
Filing Date:	16-OCT-2020
Time Stamp:	11:13:57
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-008CIPCON6_2021-09-03_SuppIDS_Trans.pdf	50039 47a1c864f1d8d8396ee7d6e40c43fbe2f560a5a5	no	2

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	REGN-008CIPCON6_2021-09-03_SuppIDS_SB08A.pdf	36149 75ceee09fb8d4e946bf0108cc51d8bcaf2eb841c	no	2
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Non Patent Literature	125387lbl-20111118.pdf	793050 5c03734e77f1148b0d55d3ab6349462c3e7a5b62	no	15
Warnings:					
Information:					
4	Non Patent Literature	Cao_2002.pdf	36299 e693fe75b60971f6ca8e1d8e35b729fc75be93b8	no	2
Warnings:					
Information:					
5	Non Patent Literature	Cao_2005.pdf	43551 eb7955ce21b031deb532a5f38d0a738efc0b1925	no	2
Warnings:					
Information:					
6	Non Patent Literature	Cursiefen_2004_Inhibition_of_hema.pdf	1178915 c20e10253dcf5992806ef217c653c91af9a56cfd	no	8
Warnings:					
Information:					
7	Non Patent Literature	Cursiefen_2004_VEGF-A_stimulates_lymphangiogenesis.pdf	4515931 d5cb6606895155d33ba1c3d3c5de79049047e84c	no	12
Warnings:					
Information:					
8	Non Patent Literature	IPR2021-00880-2021-05-05_01_Petition_for_Review_of_US9669069_880.pdf	1454636 3eb7d60c61a299ac49257bbaa2f37a6efb840229	no	91
Warnings:					
Information:					

9	Non Patent Literature	IPR2021-00880-2021-08-16_10_POPR_Mylan_069_Patent.pdf	767215 62b60957cb794ccdcc7ab77457f5d4c185c699af	no	70
Warnings:					
Information:					
10	Non Patent Literature	IPR2021-00880-Ex_1002_Albini_Decl_880.pdf	1100357 4c924cf26f34134fb38f62959de15dc3aff6a641	no	119
Warnings:					
Information:					
11	Non Patent Literature	IPR2021-00880-Ex_1003_Gerritsen_Decl_880.pdf	6081340 ae519137a3b230b16a8818485f1e3c6284ce8a00	no	59
Warnings:					
Information:					
12	Non Patent Literature	IPR2021-00881-2021-05-05_01_Petition_for_IPR_of_9254338_881.pdf	1239630 6977365612a1c5c91994d47caf135b1152338c64	no	89
Warnings:					
Information:					
13	Non Patent Literature	IPR2021-00881-2021-08-16_10_POPR_Mylan_338_Patent.pdf	801029 3c8b306e28ac79c6dc86d9d327013014abfa8136	no	76
Warnings:					
Information:					
14	Non Patent Literature	IPR2021-00881-Ex_1002_Albini_Decl_881.pdf	23233259 309bc9a75b5ef775299bfa0ff7de17df378af1a3	no	152
Warnings:					
Information:					
15	Non Patent Literature	IPR2021-00881-Ex_1003_Gerritsen_Decl_881.pdf	5491742 61cd4ed4ce04aa2bf5a9e9147c0ffeee6a69585d	no	53
Warnings:					
Information:					

16	Non Patent Literature	IPR2021-00881-Ex2001_Do_Declaration.pdf	306073 9f39940c757a122c755015fb7978e3caf54af627	no	19
Warnings:					
Information:					
17	Non Patent Literature	Mitchell_2018.pdf	6781952 dbf07aca9b56716a91c66dedab29d1b8e2b03cde	no	9
Warnings:					
Information:					
18	Non Patent Literature	Nork_2011.pdf	2073112 0422baa35069c8a843f2285a00830fdd74646fe9	no	11
Warnings:					
Information:					
19	Non Patent Literature	PGR2021-00035-2021-01-07_02_Petition_for_PGR_of_US10828345.pdf	2787300 723df9b9c0e3571dc24ad99e395633fee94380c5	no	92
Warnings:					
Information:					
20	Non Patent Literature	PGR2021-00035-2021-04-15_06_POPR.pdf	726423 3e0210cd63d3078dd2aa8dfc142c599d4e8222b6	no	96
Warnings:					
Information:					
21	Non Patent Literature	PGR2021-00035-Ex_1003_Wu_Declaration.pdf	2852812 674123a21173ad450aac4a919ea02c7fdc99587d	no	81
Warnings:					
Information:					
22	Non Patent Literature	PGR2021-00035-Ex_2001_Do_Declaration.pdf	389537 aa9eefe29251b69326733d5b6e23289687d9eddd	no	35
Warnings:					
Information:					

23	Non Patent Literature	PGR2021-00035-Ex_2002_D_Brown_Declaration.pdf	2704458 ffb2b452babea1bd305e93c4d4257b7124a4c2dd	no	22
Warnings:					
Information:					
24	Non Patent Literature	Saishin_2003.pdf	582298 2194d1ea1d4df56a20264398ec00939c017df130	no	8
Warnings:					
Information:					
25	Non Patent Literature	Wang_2002.pdf	41117 ba3f92115f8ff1f74d748cf92929101bf40fc9bc	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	66068224	

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

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT	Attorney Docket No.	REGN-008CIPCON6
	Confirmation No.	7325
	First Named Inventor	George D. Yancopoulos
	Application Number	17/072,417
	Filing Date	October 16, 2020
	Group Art Unit	To Be Assigned
	Examiner Name	To Be Assigned
Address to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

Applicant submits 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 publications discussed herein are provided to comply with the duty to disclose in accordance with 37 C.F.R. § 1.56. However, nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed

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 documents 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-008CIPCON6.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 3 September 2021

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
Reg. No. 28,807

BOZICEVIC, FIELD & FRANCIS LLP
201 Redwood Shores Parkway, Suite 200
Redwood City, CA 94065
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

Electronic Acknowledgement Receipt

EFS ID:	44366286
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	24-NOV-2021
Filing Date:	16-OCT-2020
Time Stamp:	14:41:26
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	Non Patent Literature	NPL_74_IPR2021-00881_Institution_Decision.pdf	808628 c30305d7077772c295e5fe3f2488fd27279818e9	no	41

Warnings:

Information:	
Total Files Size (in bytes):	808628

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.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

U.S. PATENT DOCUMENTS					
		DOCUMENT NUMBER	DATE	NAME	REFERENCE PROVIDED*
	1.	US 2004/0213787 A1	2004-10-28	Sleeman *et al.*	not required per 69 Fed. Reg. 56481
	2.	US 6,833,349 B2	2004-12-21	Xia *et al.*	not required per 69 Fed. Reg. 56481
	3.	US 2004/0266688 A1	2004-12-30	Nayak	not required per 69 Fed. Reg. 56481
	4.	US 2005/0032699 A1	2005-02-10	Holash *et al.*	not required per 69 Fed. Reg. 56481
	5.	US 6,879,294 B2	2005-05-24	Davis-Smyth *et al.*	not required per 69 Fed. Reg. 56481
	6.	US 2005/0281822 A1	2005-12-22	Cedarbaum *et al.*	not required per 69 Fed. Reg. 56481
	7.	US 2006/0030000 A1	2006-02-09	Alitalo *et al.*	not required per 69 Fed. Reg. 56481
	8.	US 7,378,095 B2	2008-05-27	Cao *et al.*	not required per 69 Fed. Reg. 56481
	9.	US 7,482,002 B2	2009-01-27	Cedarbaum	not required per 69 Fed. Reg. 56481
	10.	US 2009/0264358 A1	2009-10-22	Yu	not required per 69 Fed. Reg. 56481
	11.	US 7,750,138 B2	2010-07-06	Fang *et al.*	not required per 69 Fed. Reg. 56481
	12.	US 7,951,585 B2	2011-05-31	Ke	not required per 69 Fed. Reg. 56481
	13.	US 8,216,575 B2	2012-07-10	Yu	not required per 69 Fed. Reg. 56481
	14.	US 2013/0295094 A1	2013-11-07	Yancopoulos	not required per 69 Fed. Reg. 56481
	15.	US 9,657,084 B2	2017-05-23	Ke *et al.*	not required per 69 Fed. Reg. 56481

FOREIGN PATENT DOCUMENTS						
		DOCUMENT NUMBER	DATE	COUNTRY	TRANSLATION	REFERENCE PROVIDED*
	16.	CN 1304427C	2007-03-14	China	Machine translation	Herewith
	17.	CN 100502945C	2009-06-24	China	Corresponds to US 2009/0264358 A1	Herewith
	18.	CN 100567325C	2009-12-09	China	Machine translation	Herewith
	19.	WO 2012/097019	2012-07-19	WIPO	N/A	Herewith
	20.	CN 102233132 B	2013-10-23	China	Machine translation	Herewith
	21.	CN 102380096 B	2014-04-30	China	Machine translation	Herewith
	22.	CN 103212075 B	2017-06-27	China	Machine translation	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

FOREIGN PATENT DOCUMENTS						
		DOCUMENT NUMBER	DATE	COUNTRY	TRANSLATION	REFERENCE PROVIDED*
	23.	CN 107115294 A	2017-09-01	China	Machine translation	Herewith

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	24.	Anonymous, Meeting Archive Titled "PA003 Eighteen-Month Results From an Extension Study of a Phase 2, Dose- and Interval-Ranging Study of VEGF Trap-Eye in Wet AMD," presented by David S Boyer, MD at Moscone Center (October 2009)	Herewith
	25.	Anonymous, Meeting Archive Titled "PA040 One-Year Results of the DA VINCI Study of VEGF Trap-Eye in Diabetic Macular Edema," presented by Diana V Do, MD at Orange County Convention Center (October 2011)	Herewith
	26.	Anonymous, Meeting Archive Titled "PA080 One-Year Results of a Phase 2 Study of Intravitreal VEGF Trap-Eye in Patients with Neovascular Age-Related Macular Degeneration," presented by David S Boyer, MD at Georgia World Congress Center (November 2008)	Herewith
	27.	Anonymous, Meeting Archive Titled "PO259 OCT and Fluorescein Angiography Outcomes Through 1 Year for a Phase 2 Study of Intravitreal VEGF Trap-Eye in Neovascular AMD," presented by Peter K Kaiser, MD at Moscone Center (October 2009)	Herewith
	28.	Anonymous, Meeting Archive Titled "PO260 VEGF Trap-Eye Vision-Specific Quality of Life Through 52 Weeks in Patients with Neovascular AMD in CLEAR-IT 2: A Phase 2 Clinical Trial," presented by Allen C Ho, MD at Moscone Center (October 2009)	Herewith
	29.	Anonymous, Meeting Archive Titled "PO492 One-Year Results of the VIEW 1 and VIEW 2 Studies: VEGF Trap-Eye in Wet AMD," presented by David M Brown MD at Orange County Center (October 2011)	Herewith
	30.	Anonymous, Meeting Archive Titled "PO549 The 6-Month (Primary Endpoint) Results of the Phase 3 GALILEO Study: VEGF Trap-Eye in Central Retinal Vein Occlusion," presented by Jean-Francois Korobelnik, MD at Orange County Convention Center (October 2011)	Herewith
	31.	Anonymous, Meeting Archive Titled "PO571 OCT and Fluorescein Angiographic Outcomes Through 1 Year for the Phase 2 Study of Intravitreal VEGF Trap-Eye in Neovascular AMD," presented by Quan Dong Nguyen, MD at Georgia World Congress Center (November 2008)	Herewith
	32.	Bontempo, "Preformulation Development of Parenteral Biopharmaceuticals," *Drugs and the Pharmaceutical Sciences*, 85:91-108 (1997)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	33.	Bressler, N. M. Treatment of Age-Related Macular Degeneration with Photodynamic Therapy Study Group, "Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: two-year results of 2 randomized clinical trials-tap report 2," *Arch. Ophthalmol.,* 119(2):198-207 (2001)	Herewith
	34.	Brown *et al.*, "Ranibizumab for Diabetic Macular Edema (DME): 24-Month Efficacy and Safety Results of RISE - a Phase 3 Randomized Controlled Trial," ARVO Annual Meeting Abstract, *Investigative Ophthalmology & Visual Science*, 52:6647 *(April 2011)*	Herewith
	35.	Brown *et al.*, "Sustained benefits from ranibizumab for macular edema following branch retinal vein occlusion: 12-month outcomes of a phase III study," *Ophthalmology,* 118(8):1594-2049 (2011)	Herewith
	36.	Cao *et al.*, "VEGF Trap Promotes Regression of Choroidal Neovascularization (CNV) and Inhibits Fibrosis and Inflammation in the Subretinal Matrigel CNV Model," ARVO Annual Meeting Abstract, *Investigative Ophthalmology & Visual Science,* 50:2979 (April 2009)	Herewith
	37.	Center for Drug Evaluation and Research Application Number: 21-756 Medical Review(s) (December 17, 2004) <URL:https://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-756_Macugen_medr.pdf>	Herewith
	38.	Center for Drug Evaluation and Research BLA Application Number: 125156 Medical Review, (June 2006) <URL:https://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/125156s0000_Lucentis_MedR.pdf>	Herewith
	39.	Cheung *et al.*, "Combined anti-PIGF and anti-VEGF Therapy Ameliorates Pathological Neovascularization and Improves Retinal Revascularization in the Murine Model of Oxygen Induced Ischemic Retinopathy," ARVO Annual Meeting Abstract, *Investigative Ophthalmology & Visual Science*, 52:6064 (April 2011)	Herewith
	40.	Dixon *et al.*, "VEGF Trap-Eye for the treatment of neovascular age-related macular degeneration," *Expert Opin. Investig. Drugs*, 18(10):1573-1580 (2009)	Herewith
	41.	The Eyetech Study Group, "Anti-Vascular Endothelial Growth Factor Therapy for Subfoveal Choroidal Neovascularization Secondary to Age-related Macular Degeneration," *Ophthalmology*, 110(5):979-986 (May 2003)	Herewith
	42.	Heier *et al.*, "Ranibizumab for Choroidal Neovascularization Secondary to Causes Other Than Age-Related Macular Degeneration: A Phase I Clinical Trial," *Ophthalmology*, 118(1):111-118 (January 2011)	Herewith
	43.	Heier, "Intravitreal Aflibercept for Diabetic Macular Edema: 148-Week Results from the VISTA and VIVID Studies," *Ophthalmology*, 123(11):2376-2385 (2016)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	44.	Herceptin label, September 1998	Herewith
	45.	Information from ClinicalTrials.gov archive on the VIEW 2 study (NCT00637377) "VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD (VIEW 2)," v1 (March 17, 2008)	Herewith
	46.	Ip *et al.*, "A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with observation to treat vision loss associated with macular edema secondary to central retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 5," *Arch. Ophthalmol.,* 127(9):1101-1114 (2009)	Herewith
	47.	Kaiser, "Vascular endothelial growth factor Trap-Eye for diabetic macular oedema," *Br. J. Ophthalmol.*, 93(2):135-36 (February 2009)	Herewith
	48.	Korobelnik *et al.*, "Intravitreal Aflibercept Injection for Macular Edema Resulting from Central Retinal Vein Occlusion," *Ophthalmology,* 121(1):202-208 (2014)	Herewith
	49.	Krzystolik *et al.*, "Prevention of Experimental Choroidal Neovascularization With Intravitreal Anti-Vascular Endothelial Growth Factor Antibody Fragment," *Arch. Ophthalmol.*, 120(3):338-346 (Mar. 2002)	Herewith
	50.	Lalwani, "All About PrONTO: Study Yielded Good Results in AMD With Treatment Guided by OCT," *Retina Today* (May 2007)	Herewith
	51.	Lobov *et al.*, "VEGF Trap Treatment Regresses Pathological Neovessels, Improves Revascularization and Reduces Retinal Ischemia in the Murine Oxygen-Induced Retinopathy (OIR) Model," ARVO Annual Meeting Abstract, *Investigative Ophthalmology & Visual Science*, 52:3128 (April 2011)	Herewith
	52.	Lucentis Approval (June 30, 2006)	Herewith
	53.	Lucentis Label Title, 7 pages, 06/2010 [Cited in Third Party Observations filed in parent application USSN 16/055,847 for which a copy is unavailable on PAIR]	Herewith
	54.	Macular Photocoagulation Study Group, "Laser photocoagulation of subfoveal neovascular lesions in age-related macular degeneration. Results of a randomized clinical trial," *Arch. Ophthalmol.,* 109(9):1220-1231 (1991)	Herewith
	55.	Mitchell *et al.*, "Ranibizumab (Lucentis) in Neovascular Age-Related Macular Degeneration: Evidence from Clinical Trials," *Brit. J. Ophthalmology,* 94:2-13 (2010) (first online publication on May 20, 2009)	Herewith
	56.	Mitra *et al.*, "Review of anti-vascular endothelial growth factor therapy in macular edema secondary to central retinal vein occlusions," *Expert Review in Ophthalmol.*, Taylor & Francis, GB 6(6):623-629 (January 2011)	Herewith
	57.	Mousa and Mousa, "Current Status of Vascular Endothelial Growth Factor Inhibition in Age-Related Macular Degeneration," *Biodrugs*, 24(3):183-194 (2010)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	58.	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*, 113(9):1522e1-1522e14 (Sept 2006) (epub July 28, 2006)	Herewith
	59.	Regeneron Pharmaceuticals Inc., "Regeneron Receives $20 Million Milestone Payment for Initiation of Phase 3 Study of VEGF Trap-Eye in Wet AMD," Media Release: 13 Aug 2007. Available from URL: http://www.regeneron.com	Herewith
	60.	Regeneron Pharmaceuticals Inc., "An Exploratory Study of the Safety, Tolerability and Biological Effect of a Single Intravitreal Administration of VEGF Trap in Patients with Diabetic Macular Edema," poster presented at the 2007 Association for Research in Vision and Ophthalmology meeting in Ft. Lauderdale, Florida (May 2007)	Herewith
	61.	Regeneron Pharmaceuticals Inc., "CLEAR-IT-2: Interim Results Of The Phase II, Randomized, Controlled Dose-and Interval-ranging Study Of Repeated Intravitreal VEGF Trap Administration In Patients With Neovascular Age-related Macular Degeneration (AMD)," poster presented at the 2007 Association for Research in Vision and Ophthalmology meeting in Ft. Lauderdale, Florida (May 2007)	Herewith
	62.	Regeneron Pharmaceuticals Inc., "Optical Coherence Tomography Outcomes of a Phase 1, Dose-Escalation, Safety, Tolerability, and Bioactivity Study of Intravitreal VEGF Trap in Patients with Neovascular Age-Related Macular Degeneration: The CLEAR-IT 1 Study," poster presented at the 2007 Association for Research in Vision and Ophthalmology meeting in Ft. Lauderdale, Florida (May 2007)	Herewith
	63.	Regeneron Pharmaceuticals Inc., "Regeneron Reports First Quarter 2008 Financial and Operating Results," Press release May 1, 2008.	Herewith
	64.	Regeneron Pharmaceuticals Inc., Form 10-Q, published November 7, 2007, for the period ending September 30, 2007.	Herewith
	65.	Regillo *et al.*, "Randomized, Double-Masked, Sham-Controlled Trial of Ranibizumab for Neovascular Age-related Macular Degeneration: OIER Study Year 1," *American Journal of Ophthalmology*, 145(2):239-248 (2008)	Herewith
	66.	Rosenfeld *et al.*, "Optical coherence tomography findings after an intravitreal injection of bevacizumab (avastin) for neovascular age-related macular degeneration," *Ophthalmic. Surg. Lasers Imaging*, 36(4):331-335 (2005)	Herewith
	67.	Scott *et al.*, "A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with standard care to treat vision loss associated with macular Edema secondary to branch retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 6," *Arch. Ophthalmol.*, 127(9):1115-1128 & 127(12):1653 (2009)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	68.	Simo and Hernandez, "Advances in Medical Treatment of Diabetic Retinopathy," *Diabetes Care*, 32(8):1556-1562 (August 2009)	Herewith
	69.	Slides for the 2008 Retina Society Meeting "VEGF Trap-Eye in Wet AMD CLEAR-IT 2: Summary of One-Year Key Results," September 28, 2008.	Herewith
	70.	Tolentino *et al.*, "One-year Results Of The Da Vinci Study of VEGF Trap-Eye In DME," ARVO Annual Meeting Abstract, *Investigative Ophthalmology & Visual Science*, 52:6646 (April 2011)	Herewith
	71.	van Bruggen *et al.*, "VEGF antagonism reduces edema formation and tissue damage after ischemia/reperfusion injury in the mouse brain," *The Journal of clinical investigation*, 104(11):1613-1620 (1999)	Herewith
	72.	WHO Drug Information, "International Nonproprietary Names for Pharmaceutical Substances (INN)," 20(2):115-119 (2006)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

NON-PATENT LITERATURE DOCUMENTS - INSTITUTION DECISIONS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	73.	IPR2021-00880 dated November 10, 2021, for US 9,669,069 B2	Herewith
	74.	IPR2021-00881 dated November 10, 2021, for US 9,254,338 B2	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

Notice

ABSTRACT CN1304427C

[0001]

13 The present invention relates to a fusion protein that inhibits angiogenesis and its use. The fusion protein of the present invention is obtained by fusing FLT-1 and KDR fragments and immunoglobulin Fc fragments, and is called FP1, FP2, FP3, FP4, FP5 , FP6, the amino acid FLT-1 D2, FLT-1 D4, KDR, D1, KDR D3, KDR D4, FP3 sequence of FLT-1 and KDR immunoglobulin-like regions are shown in Sequence Table 1-6, FP3 coding DNA sequence See sequence table 7.

CN1304427C Angiogenesis inhibiting fusion protein and its use

Technical field

The invention relates to a series of gene recombinant proteins that can effectively inhibit angiogenesis. Angiogenesis refers to the process of growing new blood vessels from existing blood vessels. The vast majority of blood vessels in adults are at rest, and angiogenesis is only seen in a few pathological or physiological states, such as tumors, diabetic lesions, arthritis, anemia organs, and endometrial hyperplasia. In the process of tumor occurrence, angiogenesis plays a key role in the rapid growth of tumors (Hanahan and Folkman: Patterns andemerging mechanisms of the angiogenic switch during tumorigenesis. Cell. 1996, 86: 353-364). Research on animal tumor models and human clinical trials have shown that inhibiting the formation of neovascularization in tumors can effectively prevent the growth and development of tumors, thereby extending the life of patients

Angiogenesis is regulated and controlled by a variety of biologically active substances. The main cells that dominate the angiogenesis process are the vascular endothelial cells that make up the innermost layer of the blood vessel wall. Various growth factors can bind to the corresponding receptors on the surface of vascular endothelial cells, and regulate the activity of vascular endothelial cells through the intracellular signal transmission system, thereby regulating the angiogenesis.

Background technique

Among various growth factors, vascular endothelial growth factor (Vascular endothelial cell growth factor, VEGF) is the most important factor regulating angiogenesis (Ferrara: VEGF and the question for tumor angiogenesis factor. Nat. Rev. Cancer, 2002, 10: 795-803. Ferrara: Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. Semin. Oncol. 2002, 29 (6sumppl): 10-14). Vascular endothelial growth factor can be secreted by a variety of cells, but it is often overexpressed in tumor cells. Endothelial growth factor works by binding to the corresponding receptor. There are two main receptors that bind to VEGF: FLT-1 and KDR. In terms of molecular structure, these two receptors are composed of three different functional regions. The first functional area is the extracellular part located outside the cell. It consists of seven immunoglobulin-like regions (d1-d7). This part has specific affinity for VEGF and is a key part of VEGF binding to the receptor. The second functional area is the part of the transmembrane composed of hydrophobic amino acids. The third functional area is the intracellular part, which includes the tyrosine kinase group. After the receptor is activated by VEGF, the tyrosine kinase group is phosphorylated, thereby initiating the signal transmission system inside the cell, and ultimately causing functional changes in endothelial cells, leading to angiogenesis.

FLT-1 and KDR are mainly distributed in vascular endothelial cells. Therefore, VEGF has a highly specific regulatory effect on vascular endothelial cells. VEGF has the functions of promoting endothelial cell division, guiding endothelial cell migration, inhibiting cell apoptosis, and inducing vascular morphogenesis, etc. It is a highly effective inducer of angiogenesis.

In tumor tissues, the expression level of VEGF is higher than in normal tissues. In addition, the rapid growth of tumors often leads to hypoxia inside the tumors. The decrease in oxygen partial pressure leads to an increase in VEGF expression. Therefore, VEGF is a key factor leading to angiogenesis in tumors. Many animal experiments have shown that blocking the binding of VEGF to its receptor can effectively inhibit angiogenesis in tumors, thereby preventing tumor growth. In other diseases related to angiogenesis, such as visual retinopathy of diabetes and arthropathy of arthritis, etc., VEGF is also closely related to the development of these diseases.

In view of the key role of VEGF in tumors and other diseases, proteins or compounds that can specifically block VEGF will have the potential to treat these diseases. For example, studies have shown that anti-VEGF neutralizing antibodies can effectively inhibit tumor growth (Jain: Tumor angiogenesis and accessibility: role of vascular endothelial growth factor. Semin. Oncol. 2002, 29 (6 supup): 3-9). Therefore, it is of great significance to find new and more effective VEGF blockers. Because FLT-1 and KDR have a natural affinity for VEGF,

studies have investigated the anti-angiogenic effects of soluble FLT-1 (the extracellular portion of FLT-1) or soluble KDR (the extracellular portion of KDR). Soluble FLT-1 can effectively inhibit the proliferation of vascular endothelial cells in vitro, but its half-life in serum is very short, and it cannot reach an effective serum concentration. Soluble KDR can also inhibit the proliferation of vascular endothelial cells in vitro, but its antitumor effect is not good in animal experiments. Part of the basic amino acids in the third immunoglobulin-like region of FLT-1 is the main cause of FLT-1 instability in the body, so replacing these basic amino acids with partial amino acids of KDR can increase the stability of FLT-1.

US patents 6100701, 5952199, and 6383486 describe several proteins fused with partial fragments of KDR and partial fragments of FLT-1, but due to their instability and large side effects, no further development has been made.

Summary of the invention

The invention relates to a method for constructing and producing protein drugs capable of blocking VEGF using genetic engineering technology and the application of such drugs in disease treatment.

The invention provides six fusion proteins obtained by fusing fragments of FLT-1 and KDR and immunoglobulin Fc fragments, which have the biological effect of blocking vascular endothelial cell growth factor and inhibiting angiogenesis, referred to as FP1, FP2, FP3 , FP4, FP5, FP6, with the following structure:

a. FP1 is a protein fused by the second immunoglobulin-like region of FLT-1 and the third immunoglobulin-like region of KDR: FLTd2-KDRd3-Fc;

b. FP2 is the fusion protein of the first immunoglobulin-like region of KDR, the second immunoglobulin-like region of FLT-1 and the third immunoglobulin-like region of KDR: KDRd1-FLTd2-KDRd3-Fc

c. FP3 is a protein fused by the second immunoglobulin-like region of FLT-1 and the 3-4 immunoglobulin-like region of KDR: FLTd2-KDRd3, 4-Fc;

d. FP4 is a protein fused by the second immunoglobulin-like region of FLT-1, the third immunoglobulin-like region of KDR and the fourth immunoglobulin-like region of FLT-1: FLTd2-KDRd3-FLTd4-Fc ;

e. FP5 is a protein fused by the second immunoglobulin-like region of FLT-1 and the third 3-5 immunoglobulin-like region of KDR: FLTd2-KDRd3, 4, 5-Fc;

f. FP6 is a protein fused by the second immunoglobulin-like region of FLT-1, the third immunoglobulin-like region of KDR and the 4-5 immunoglobulin-like region of FLT-1: FLTd2-KDRd3-FLTd4 , 5-Fc.

The amino acid FLT-1D2, FLT-1D4, KDR, D1, KDR D3, KDR D4, and FP3 sequences of the above FLT-1 and KDR immunoglobulin-like regions are shown in Sequence Table 1-6, and the FP3 coding DNA sequence is shown in Sequence Table 7.

Where FLT stands for FLT-1 sequence, KDR stands for KDR sequence, d is the immunoglobulin-like region of FLT-1 or KDR, which can also be represented by a capital D, and Fc is the human immunoglobulin Fc sequence.

The immunoglobulin FC fragment is selected from human immunoglobulin FC or animal immunoglobulin FC. Such as: IgG, IgM, IgA or subtypes IgG1, IgG2, IgG3, IgG4. The immunoglobulin FC fragments are full-length FC or partial FC sequences, selected from CH2 fragments, CH3 fragments, and stranded region fragments. These sequences and fragments are all existing technologies and can be found in textbooks.

The present invention also provides the application of the fusion protein of the present invention in the preparation of a drug that blocks the biological action of vascular endothelial cell growth factor and inhibits angiogenesis.

The key to this invention is to construct a series of fusion proteins fused with different FLT-1 fragments, KDR fragments, and human immunoglobulin Fc according to the structural design of FLT-1 and KDR, and then use VEGF binding test, etc. Methods Screen fusion proteins with the greatest affinity for VEGF. So as to get the

best VEGF blocker. The construction technology of fusion protein is based on molecular cloning method. For specific experimental methods, please refer to the second and third editions of Molecular Cloning.

According to the amino acid sequence structure of each region of FLT-1 and KDR molecules, among the above fusion proteins, the FP1 fusion protein will provide the basic structure that binds to VEGF, which is composed of the second immunoglobulin region of FLT-1 (FLTd2) Sequence, the third immunoglobulin region (KDRd3) sequence of KDR and Fc. In the FP2 fusion protein, the amino acid sequence from the first immunoglobulin-like region (KDRd1) of KDR is added. These sequences can increase the site of binding to VEGF, thereby increasing the affinity for VEGF. The sequence of the fourth (FLTd4 or KDRd4) immunoglobulin-like region of FLT-1 or KDR was added to the FP3 and FP4 fusion proteins. FP5 and FP6 are the fourth and fifth immunoglobulin-like regions (FLT-1d4, 5, KDRd4, 5) with the addition of FLT-1 or KDR on the basis of FP1. These newly added sequences will facilitate the coupling between the fusion proteins, thereby further forming a spatial structure that is conducive to binding to VEGF and increasing the affinity for binding to VEGF.

The fusion protein of the present invention can be obtained by gene recombination technology, which is a conventional technology. First, the DNA encoding the above-mentioned fusion protein is obtained. The DNA can be obtained by conventional techniques, such as PCR synthesis, etc. Carrier. The vector used may be a plasmid, virus or DNA fragment commonly used in molecular biology. The protein secretion signal sequence is added before the amino terminal of each fusion protein to ensure that the protein is secreted from the cell. The vector sequence includes a promoter for driving gene expression, protein translation initiation and termination signals, and polyadenylation (PolyA) sequence. The vector has an antibiotic resistance gene to facilitate the propagation of the plasmid in bacteria. In addition, the vector also includes eukaryotic cell selection genes for the selection of stable transfected cell lines.

Since there is no absolute boundary between the amino acid sequences of each immunoglobulin-like region in FLT-1 and KDR, the length of the amino acid sequence of each immunoglobulin-like region may vary to some extent. Therefore, the amino acid sequence of the fusion protein involved in the present invention may also have certain changes. They all fall within the scope of the present invention.

After completing the construction of the plasmids for the various fusion proteins listed above, the plasmid DNA can be used to transfect the cells and express the corresponding proteins. There are many expression systems that can be used to express these fusion proteins, including (but not limited to) mammalian cells, bacteria, yeast, insect cells, etc. (where mammalian cells and insect cells are eukaryotic cells, bacteria and yeast The cells are prokaryotic cells. The proteins expressed from mammalian cells have glycosylation. Since the amino acid sequence of the fusion protein of the present invention includes glycosylated amino acids, mammalian cells are the best cells for expressing these proteins. There are many mammalian cells that can be used for large-scale protein expression, such as 293 cells, CHO cells, SP20 cells, NS0 cells, COS cells, BHK cells, PerC6 cells, etc. Many other cells can also be used for the expression and expression of these proteins Production is therefore included in the list of cells that can be used in the present invention. Plasmids encoding polypeptides can be transfected into cells. There are many methods for transfecting cells, including (but not limited to): electrodrill (electroporation), liposome (liposome) mediated, calcium mediated, and so on.

In addition to mammalian cells, other expression systems can also be used for the expression of these polypeptides, such as bacteria, yeast, insect cells, etc. They are also included in the cells that can be used in the present invention. The protein production of these expression systems is higher than that of mammalian cells. However, the expressed proteins lack glycosylation or form different sugar chains than mammalian cells.

After the fusion protein is expressed, the concentration of the fusion protein in the cell culture fluid can be determined by enzyme-linked immunosorbent assay (ELISA) or other methods. Since these fusion proteins have immunoglobulin Fc fragments, protein A affinity chromatography can be used to extract the expressed fusion protein.

In the present invention, various fusion proteins are obtained from plasmid-transfected 293 cell culture fluid. The VEGF binding test was then used to compare the affinity of various proteins for VEGF. Furthermore, human vascular endothelial cell division test induced by VEGF was used to detect and compare the blocking effect of each fusion protein on VEGF. The experimental results prove that the various fusion proteins constructed by the present invention have a high affinity for VEGF (see FIG. 2), and they can effectively block the stimulation of vascular endothelial cells by VEGF and inhibit the division of endothelial cells. Further experiments found that FP3 had the best blocking effect on VEGF. It is the most effective fusion protein to block VEGF.

Therefore, the fusion protein constructed by the present invention has a blocking effect on VEGF. The molecular weight of FP1 is the smallest. FP3 is most effective in blocking VEGF. These fusion proteins have the biological properties of anti-angiogenesis, and thus have the possibility of treating diseases.

Since the basic function of the various fusion proteins of the present invention is to block VEGF, these proteins may be used in diseases related to angiogenesis or VEGF. These diseases may include (but are not limited to) various tumors, optic retinopathy, arthritis, etc. The fusion protein can be injected into the patient as an extracted recombinant protein. The DNA sequence of the fusion protein can also be inserted into an appropriate vector and expressed in the patient by gene therapy or cell therapy. Therefore, there are many ways to use the fusion protein according to the present invention, including not only the protein itself but also the DNA encoding the fusion protein.

In order to further prove the anti-vascular effect of the fusion protein in vivo, the present invention tested the anti-tumor effect of the FP3 fusion protein in animals. In mouse B16F10 melanoma and xenograft model human PC-3 prostate cancer, the fusion protein very effectively inhibited the growth of the tumor and prolonged the life of the individual. Therefore, the fusion protein constructed by the present invention has high anti-cancer ability.

The invention also includes a pharmaceutical composition containing the fusion protein of the invention, which may contain a pharmaceutically acceptable carrier. The composition can exist in any form of pharmaceutical preparation, preferably an injection, and most preferably a freeze-dried injection. The pharmaceutical composition in the form of a pharmaceutical preparation can be prepared according to conventional techniques in pharmaceutics, including the pharmaceutical active ingredient, The fusion protein is mixed with the drug carrier, and the required dosage form is prepared according to the conventional technique of pharmaceutics.

Compared with the prior art, the fusion protein of the present invention has obvious advantages, good stability, high yield, and low side effects. The particularly preferred fusion protein of the present invention is FP3, which is obtained through extensive screening. Comparative experiments on other fusion proteins invented and other similar fusion proteins in the prior art revealed that the fusion protein has particularly excellent antitumor effects in animals. In mouse B16F10 melanoma and xenograft model human PC-3 prostate cancer, the FP3 fusion protein very effectively inhibits the growth of the tumor and prolongs the life of the individual. Experiments have shown that FP3 is more effective than other fusion proteins and has side effects It is smaller and has the best stability after preparation. Among the fusion proteins of the present invention, it has the most anti-cancer application value.

BRIEF DESCRIPTION

Figure 1 shows the structural composition of the six fusion proteins. They are constructed from different FLT-1 and KDR polypeptide fragments and immunoglobulin Fc fragments by genetic engineering.

Figure 2 shows the test results of six fusion proteins binding to VEGF. The OD reading represents the binding signal of the fusion protein to VEGF. The results show that they have a strong binding capacity with VEGF, especially FP3 has the strongest binding capacity.

Figure 3 The fusion protein effectively inhibits the division of human vascular endothelial cells in vitro.

Figure 4 shows that the fusion protein FP3 effectively inhibits the growth of mouse B16F10 melanoma in vivo.

Figure 5 shows that the fusion protein effectively inhibits the growth of human PC-3 prostate cancer in mice.

Figure 6 A comparative study of the fusion polypeptides FP1 and FP3 effectively inhibiting the growth of mouse tumors.

detailed description

The following examples illustrate the construction, testing and application of the fusion proteins involved in the present invention. However, the content and use of the present invention are not limited to the scope of examples.

Example 1: Construction of fusion protein and its plasmid.

In addition to immunoglobulin Fc fragments, the original sequences for constructing various fusion proteins in the present invention are derived from the corresponding cDNAs of FLT-1 and KDR. Since the expression of FLT-1 and KDR are mainly found in vascular endothelial cells, the present invention uses RNA purification kit (QIAGEN) to extract total RNA from human umbilical vein vascular endothelial cells (HUVEC). Reverse transcriptase is then used to synthesize cDNA from RNA. Then use different primers to obtain the desired FLT-1 and KDR fragments by polymerase chain reaction (PCR) amplification. Finally, PCR was used to fuse sequences from FLT-1, KDR, and human immunoglobulin Fc (IgG1 Fc) to construct DNA sequences of different fusion proteins. The structure of the six fusion proteins is shown in Figure 1.

Construction of FP3 gene:

Human umbilical vein endothelial cells (HUVEC cells) (Clonetics) were cultured in T-175 flasks with EGM-2 medium (Clonetics). Collect about 1 × 10e7 cells, use Qiagen's RNA extraction kit to extract the total RNA of the cells, and use the Invitrogen cDNA kit to synthesize cDNA, and store at -80 ° C until use. The following specific primers were used to amplify FLT-1 and KDR gene fragments from HUVEC cDNA.

Human IgG1 Fc-specific primers were used to amplify IgG1 Fc gene fragments from cDNA derived from lymph nodes (BD Clontech).

Primers:

FLT-1 D2 forward: cctttcgtagagatgtacagtga

FLT-1 D2 reverse: tatgattgtattggtttgtccat

KDR D3-4 forward: gatgtggttctgagtccgtctca

KDR D3-4 reverse: cggtgggacatacacaaccaga

Human IgG1 Fc forward: gacaaaactcacacatgcccact

Human IgG1 Fc reverse: tcatttacccggagacagggagag

Under the conditions of denaturation 95 °C, 30 minutes, annealing 56 °C, 45 seconds, extension 72 °C, 2 minutes, PCR amplification was performed for 30 cycles to obtain FLT-1 and KDR IgG-like domain PCR products and human IgG1 Fc segment PCR product. Using TA cloning kit, clone the PCR product into pCR2.1 plasmid and transfect E. coli, select white colonies, add LB medium, and culture overnight. Qiangen Plasmid Extraction Kit extracts plasmids, digests them, and performs sequencing identification.

Using splicing PCR (sewing PCR) method, FLT-1, KDR and IgG Fc cDNA were linked together, and EcoRI enzyme digestion site was designed in the primer. After digestion with EcoRI, Qiangen purification kit purified DNA fragment and inserted pcDNA3.1 plasmid, recombinant plasmid transfected E. coli, select positive colonies, add LB medium, and culture overnight. Qiagen Plasmid Extraction Kit extracts the plasmid, digests it and performs sequencing identification. The confirmed plasmid was then transfected into 293 or CHO cells to obtain a cell line stably expressing FP3. The specific amino acid sequence of FP3 is shown in Sequence Listing 6,

Example 2: Expression of fusion protein in cells.

One of the components of the present invention is to express the constructed fusion protein in cells. After completing the construction of each plasmid, high-purity plasmid DNA was extracted using a plasmid DNA purification kit (QIAGEN). Then, the plasmid DNA was introduced into 293 cells using a FUGEN6 plasmid transfection kit (ROCHE Corporation). According to the required protein mass, two different plasmid transfection methods were used to express the fusion protein.

The first method is the transient transfection method, which can be used to obtain small-scale fusion proteins. First, 293 cells were cultured in cell culture dishes with DMEM complete medium containing 10% fetal bovine serum. When the cells grow to cover 60-80% of the area, the complex of plasmid DNA and FUGEN6 reagent is added to the cell culture fluid. The next day, the medium was replaced with serum-free DMEM medium.

Continue culturing for another three days, and then collect the supernatant. These supernatants contain fusion protein polypeptides expressed from cells. The concentration of fusion polypeptide was quantitatively determined by ELISA. In this way, the present invention expresses the above six fusion polypeptides.

The second method is to use stable transfection (Stable transfection) method to establish stable cells to express a large number of fusion protein polypeptides. The cells used were also 293 cells. The plasmid transfection method is the same as the transient transfection method described above. However, on the second day of transfection, the cells are cloned and cultured in a limited density dilution method in DMEM containing neoenzyme. After about 21 days, the new enzyme-resistant clones were picked and the cells were expanded and cultured. Finally, the cells are cultivated in drum culture flasks to produce fusion polypeptides. The concentration of fusion polypeptide was quantitatively determined by ELISA.

The invention proves that the constructed fusion polypeptide can be expressed and produced from the plasmid through cell transfection.

Example 3: Binding experiment of fusion polypeptide and VEGF.

In the present invention, the VEGF binding test was used to determine the binding ability of each fusion polypeptide to VEGF. In this test, the recombinant VEGF (Chemicom) protein was first coated on a 96-well ELISA plate. Then use 5% milk powder solution to block non-specific protein binding sites. Then add various fusion proteins containing different concentrations to each well and incubate at 37 degrees for two hours. After washing, rabbit anti-human Ig antibody-HRP was added. Finally, the color is developed with peroxidase substrate. OD value of each well of 96-well plate was measured with ELISA reader. A high OD value represents the binding signal of the fusion protein to VEGF.

As shown in FIG. 2, the six fusion proteins constructed and expressed by the present invention all have the ability to bind to VEGF. At a concentration of 1 nanogram per milliliter, their binding signal to VEGF can be detected. Therefore, these six fusion proteins have a high affinity for VEGF. However, in comparison, FP3 has the largest binding capacity to VEGF and is the most powerful VEGF blocker. Its half maximum binding concentration (Halfmaximal binding concentration) is about 5 times lower than FP1. The binding ability of FP5 to VEGF is slightly weaker than FP3. This result indicates that the amino acid sequence of the fourth immunoglobulin-like region of KDR can enhance the fusion protein's ability to block VEGF. However, further increasing the sequence of KDR in the fusion protein, such as the fifth immunoglobulin-like region, does not further increase the ability to inhibit VEGF. The inhibitory ability of the other three fusion proteins is weaker than FP3 and FP5, but stronger than FP1.

Example 4: The fusion protein effectively inhibits the division of human vascular endothelial cells in vitro.

Another key example of the present invention is to prove that the constructed fusion protein can effectively block the division of vascular endothelial cells induced by VEGF. In this experiment, 96-well cell culture plates were seeded with umbilical vein endothelial cells (HUVEC cells, CLONETICS). The cell culture medium is EBM basic medium (CLONETICS), containing 2% fetal bovine serum and 15ng / ml VEGF. In the cell culture fluid of the experimental group, 293 cell supernatant containing fusion proteins of different concentrations was added. In the cell culture fluid of the negative control group, supernatant of 293 cells (without fusion protein) without plasmid transfection was added. HUVEC cells of different treatments were continuously cultured at 37 degrees. After three days, the cell count determines the HUVEC density in each well.

As shown in Figure 3, the six proteins can effectively block the stimulation of VEGF on their receptors. Among them, FP3 had the most obvious inhibitory effect on HUVEC growth.

The HUVEC cell division test shows that the six fusion proteins constructed by the present invention can inhibit the division of vascular endothelial cells. Since the division of HUVEC cells in this experiment is caused by VEGF stimulation, these six proteins can effectively block the stimulation of VEGF on their receptors. They all have the function of inhibiting angiogenesis. Among these six proteins, FP3 has the strongest inhibitory effect on HUVEC cell division, and their half-inhibitory concentration (IC50) is around 3 ng / ml. The IC50 of FP1 is around 12 ng / ml. The IC50 of FP2, FP4, FP5, and FP6 is 5-8ng / ml.

Example 5: Fusion polypeptide can effectively inhibit the growth of mouse tumors.

As a VEGF blocker, one of the applications of the fusion protein constructed in the present invention is for the treatment of tumors. In view of the high-efficiency blocking effect of FP3 fusion protein on VEGF, the present invention selects FP3 to conduct an anti-tumor effect test in animals.

The mouse tumor model tested in the present invention is B16F10 melanoma. This is a rapidly growing malignant tumor. In this experiment, B16F10 cells were injected into the back of the mouse. The purified fusion protein was injected via tail vein. The injection dose is 400 micrograms per mouse (the average body weight of mice is about 22 grams) twice a week. The control group was injected with the same dose of purified human immunoglobulin Fc. Figure 4 shows the growth curve of the tumor. The fusion protein very effectively inhibited the growth of this melanoma (P (0.01).

The xenograft model that allows human tumor cells to grow in nude mice is the closest animal tumor model to human tumors. Nude mice lack immune rejection ability, so many human tumor cell lines can grow in nude mice to form tumors. The present invention tested the inhibitory effect of fusion protein FP3 on the growth of human prostate cancer PC-3 cells in nude mice. In this model, PC-3 cells were injected into the back of nude mice and the purified fusion protein was injected from the tail vein. Each mouse is 400 μg each time, twice a week. The control group was injected with the same dose of human immunoglobulin Fc. The experimental results are shown in Figure 5. In the control group, 45 days after tumor cell inoculation, the tumor had grown to greater than 1000 mm3. In animals injected with fusion protein, the fusion protein almost completely inhibited the growth of PC-3 tumors (P (0.01), and had a very significant therapeutic effect on tumors.

Example 6: Comparative study of fusion polypeptides FP1 and FP3 effectively inhibiting the growth of mouse tumors.

In order to better illustrate that FP3 has a good tumor suppressing effect, FP1 and FP3 were selected as a comparative test for inhibiting tumor growth. Ten well-grown nude mice were selected. Each glioma C6 cell 1 × 105, 0.05 ml was injected into the back, and the purified fusion proteins FP1 and FP3 were injected into the tail vein, 2.5 mg / kg, twice a week. To 31 days. The control group was injected with the same dose of human immunoglobulin Fc. The experimental results are shown in Figure 6. FP1 and FP3 have a very significant therapeutic effect on the tumor. By day 35, the tumor volume of the FP1 group was 1167.3, while the FP3 group was 557.6, and the control group had reached 1312.3 by the 24th day. Therefore, the effect of FP3 is more obvious (P (0.05.). The results are shown in Figure 6.

In summary, the fusion protein constructed by the present invention has a high affinity for VEGF, can inhibit the proliferation of vascular endothelial cells in vitro, and can significantly inhibit the growth of tumors in vivo. Since angiogenesis is necessary for the proliferation of all tumors, the fusion protein of the present invention can be used for the treatment of various tumors.

Sequence Listing

(110) Chengdu Kanghong Technology Industrial (Group) Co., Ltd.

(120) Fusion protein inhibiting angiogenesis and use thereof

(160)7

(210)1

(211)93

(212) PRT

(213) Artificial sequence

(400)1

Pro, Phe, Val, Glu, Met, Tyr, Ser, Glu, Ile, Pro, Glu, Ile, Ile, His, Met, Thr, Glu, Gly, Arg, Glu

1 5 10 15 20

LeuValIleProCysArgsValThrThrPronAsIleThrThrValThrThreuLysLysLhePhePro

25 30 35 40

Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Ser Arg Lys Gly Phe Ile

45 50 55 60

Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Gln Ala Thr Val Asn Gly

65 70 75 80

His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr

85 90 93

(210)2

(211)96

(212) PRT

(213) Artificial sequence

(400)2

Phe Ile Thr Vals Lys His Arg lys Lys Gln Gln Glu Val Val Leu Glu Thr Thr Val Ala Gly Lys Arg Ser

1 5 10 15 20

Tyr Arg Leu Ser Ser Met Lys Val Lys Ala Phe Pro Pro Ser Glu Glu Val Val Trp Leu Lys Asp

25 30 35 40

Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu Thr Arg Gly Tyr Ser Leu Ile Ile

45 50 55 60

Lys, Asp, Val, Thr, Glu, Asp, Ala, Gly, Asn, Tyr, Thr, Ile, Leu, Leu, Ser, Ile, Lys, Gln, and Ser

65 70 75 80

Asn Val Phe Lys Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro

85 90 95 96

(210)3

(211)86

(212) PRT

(213) Artificial sequence

(400)3

Pro Arg Leu Ser Ser Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr Leu Gln Ile

1 5 10 15 20

Thr, Cys, Arg, Gly, Gln, Arg, Asp, Leu, Asp, Trp, Leu, Trp, Pro, Asn, Asn, Gln, Ser, Gly, Ser, Glu

25 30 35 40

Gln, Arg, Val, Glu, Val, Thr, Glu, Cys, Ser, Asp, Gly, Leu, Phe, Cys, Lys, Thr, Leu, Thr, and IlePro

45 50 55 60

Lys Val Ile Gly Asn Thr Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala

65 70 75 80

Ser Val Ile Tyr Val Tyr

85 86

(210)4

(211)102

(212) PRT

(213) Artificial sequence

(400)4

Val, Val, Leu, Ser, Pro, Ser, His, Gly, Ile, Glu, Leu, Ser, Val, Gly, Glu, Lys, Leu, Val, Leu, Asn

1 5 10 15 20

Cys Thr, Ala, Arg, Thr, Glu, Leu, Asn, Val, Gly, Ile, Asp, Phe, Asn, Trp, Glu, Tyr, Pro, Ser, and Ser

25 30 35 40

LysHisGlnHisLysLysLeuLeuValAsAsArgArgasLeuLysThrThrGlnSerGlySerGluGluMet

45 50 55 60

Lys, Lys, Phe, Leu, Ser, Thr, Leu, Thr, Ile, Asp, Gly, Val, Thr, Arg, Ser, Asp, Gln, Gly, Leu, Tyr

65 70 75 80

Thr, Cys, Ala, Ala, Ser, Ser, Gly, Leu, Met, Thr, Lys, Lys, Asn, Ser, Thr, Phe, Val, Arg, and His

85 90 95 100

GluLys

102

(210)5

(211)92

(212) PRT

(213) Artificial sequence

(400)5

Phe Val Ala Phe Gly Ser Ser Gly Met Glu Ser Ser Leu Val Glu Ala Thr Val Val Gly Glu Arg Val

1 5 10 15 20

Arg, Ile, Pro, Ala, Lys, Tyr, Leu, Gly, Tyr, Pro, Pro, Pro, Glu, Ile, Lys, Trp, Tyr, Lys, Asn, Gly

25 30 35 40

Ile Pro Leu Glu Ser Ser As Thr His Ile Lys Ala Gly His Val Val Leu Thr Ile Met Glu Val

45 50 55 60

Ser, Glu, Arg, Asp, Thr, Gly, Asn, Tyr, Thr, Val, Ile, Leu, Thr, Asn, Pro, Ile, Ser, Lys, Glu, and Lys

65 70 75 80

GlnSerHisValValSerLeuValValTyrValPro

85 90 92

(210)6

(211)552

(212) PRT

(213) Artificial sequence

(400)6

MetValSerTyrTrpAspThrThr GlyValLeuLeuCysAlaLeuLeuSerCysLeuLeuLeu

1 5 10 15 20

Thr, Gly, Ser, Ser, Ser, Gly, Gly, Arg, Pro, Phe, Val, Glu, Met, Tyr, Ser, Glu, Ile, Pro, Glu, and Ile

25 30 35 40

Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Val Thr Ser Pro As As Ile

45 50 55 60

Thr, Val, Thr, Leu, Lys, Lys, Phe, Pro, Leu, Asp, Thr, Leu, Ile, Pro, Asp, Gly, Lys, Arg, Ile, Ile

65 70 75 80

Trp, Asp, Ser, Arg, Lys, Gly, Phe, Ile, Ile, Ser, Asn, Ala, Thr, Tyr, Lys, Glu, Ile, Gly, Leu, and Leu

85 90 95 100

Thr, Cys, Glu, Ala, Thr, Val, Asn, Gly, His, Leu, Tyr, Lys, Thr, Asn, Tyr, Leu, Thr, His, Arg, Gln

105 110 115 120

Thr Asn Thr Ile Ile Asp Val Val Leu Ser Ser Pro His His Gly Ile Glu

LeuSerValGly

125 130 135 140

Glu, Lys, Leu, Val, Leu, Asn, Cys, Thr, Ala, Arg, Thr, Glu, Leu, Asn, Val, Gly, Ile, Asp, Phe, Asn

145 150 155 160

TrpGluGluTyrProSerSerLysHisGlnHisLysLysLysLeuValAsAsArgArgsAspLeuLysThr

165 170 175 180

Gln, Ser, Gly, Ser, Glu, Met, Lys, Lys, Phe, Leu, Ser, Thr, Leu, Thr, Ile, Asp, Gly, Val, Thr, Arg

185 190 195 200

Ser, Asp, Gln, Gly, Leu, Tyr, Thr, Cys, Ala, Ala, Ser, Ser, Gly, Leu, Met, Thr, Lys, Lys, Asn, and Ser

205 210 215 220

Thr, Phe, Val, Arg, Val, His, Glu, Lys, Pro, Phe, Val, Ala, Phe, Gly, Ser, Gly, Met, Glu, Ser, Leu

225 230 235 240

Val, Glu, Ala, Thr, Val, Gly, Glu, Arg, Val, Arg, leu, Pro, Ala, Lys, Tyr, Leu, Gly, Tyr, Pro

245 250 255 260

Pro Glu, Ile, Lys, Trp, Tyr, Lys, Asn, Gly, Ile, Pro, Leu, Glu, Ser, Asn, His, Thr, Ile, Lys, Ala

265 270 275 280

GlyHisValLeuThrThr IleMetGluGluSerGluGluArg AspThrThryGlyAsnTyrThrThrValIle

285 290 295 300

Leu Thr Asn Pro Ile Ser Ser Lys Glu Lys Gln Ser Ser His Val Val Ser Ser Leu Val Val Tyr Val

305 310 315 320

Pro Pro Gly Pro Pro Gly Asp Lys Thr His His Thr Cys Pro Pro Leu Cys Pro Pro Ala Pro Pro Glu Leu Leu

325 330 335 340

Gly, Gly, Pro, Ser, Val, Phe, Leu, Phe, Pro, Pro, Lys, Pro, Lys, Asp, Thr, Leu, Met, Ile, Ser, Arg

345 350 355 360

Thr, Pro, Glu, Val, Thr, Cys, Val, Val, Asp, Val, Ser, His, Glu, Asp, Pro, Glu, Val, Lys, Phe

365 370 375 380

Asn, Trp, Tyr, Val, Asp, Gly, Val, Glu, Val, His, Asn, Ala, Lys, Thr, Lys, Pro, Arg, Glu, Glu, Gln

385 390 395 400

Tyr, Asn, Ser, Thr, Tyr, Arg, Val, Val, Ser, Val, Leu, Thr, Val, Leu, His, Gln, Asp, Trp, Leu, Asn

405 410 415 420

Gly, Lys, Glu, Tyr, Lys, Cys, Lys, Val, Ser, Asn, Lys, Ala, Leu, Pro, Ala, Pro, Ile, Glu, Lys, Thr

425 430 435 440

Ile, Ser, Lys, Ala, Lys, Gly, Gln, Pro, Arg, Glu, Pro, Gln, Val, Tyr, Thr, Leu, Pro, Pro, Ser, Arg

445 450 455 460

Asp Glu Leu Thr Lys Asn Gln Val Ser Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser

465 470 475 480

Asp, Ile, Ala, Val, Glu, Trp, Glu, Ser, Asn, Gly, Gln, Pro, Glu, Asn, Asn, Tyr, Lys, Ala, Thr, Pro

485 490 495 500

Pro Val Leu Asp Ser Ser Asp Gly Ser Ser Phe Phe Leu Tyr Ser Ser Lys Leu Thr Val Asp Lys Ser

505 510 515 520

Arg, Trp, Gln, Gln, Gly, Asn, Val, Phe, Ser, Cys, Ser, Val, Met, His, Glu, Ala, Leu, His, Asn, His

525 530 535 540

Tyr Thr, Gln, Lys, Ser, Leu, Ser, Leu, Ser, Pro, Gly, Lys

545 550 552

(210)7

(211)1656

(212) DNA

(213) Artificial sequence

(400)7

atggtcagct actgggacac cggggtcctg ctgtgcgcgc tgctcagctg tctgcttctc 60

acaggatctagttccggaggtagaccttttcgtagagatgtacagtgaaatccccgaaatt120

atacacatga ctgaaggaag ggagctcgtc attccctgcc gggttacgtc acctaacatc 180

actgttactt taaaaaagtt tccacttgac actttgatcc ctgatggaaa acgcataatc 240

tgggacagtagaaagggcttcatcatatcaaatgcaacgtacaaagaaatagggcttctg300

acctgtgaag caacagtcaa tgggcatttg tataagacaa actatctcac acatcgacaa 360

accaatacaa tcatagatgtggttctgagtccgtctcatggaattgaactatctgttgga420

gaaaagcttg tcttaaattg tacagcaaga actgaactaa atgtggggat tgacttcaac 480

tgggaatacccttcttcgaagcatcagcataagaaacttgtaaaccgagacctaaaaacc540

cagtctggga gtgagatgaa gaaatttttg agcaccttaa ctatagatgg tgtaacccgg 600

agtgaccaag gattgtacac ctgtgcagca tccagtgggc tgatgaccaa gaagaacagc 660

acatttgtcagggtccatgaaaaccttttctgttgcttttggaagtggcatggaatctctg720

gtggaagcca cggtgggggg gcgtgtcaga atccctgcga aggtaccttgg ttacccaccc 780

ccagaaataaaatggtataaaaatggaatacccctgagtccaatcacacaattaaagcg840

gggcatgtac tgacgattat ggaagtgagt gaaagagaca caggaaatta cactgtcatc 900

cttaccaatc ccatttcaaa ggagaagcag agccatgtgg tctctctggggt tgtgtatgtc 960

ccaccgggcc cgggcgacaa aactcacaca tgcccactgt gcccagcacc tgaactcctg 1020

gggggaccgtcagtcttcctcttcccccaaaaaacccaaggacaccctcatgatctcccgg1080

acccctgagg tcacatgcgtggtggtggacgtgagccacg aagaccctgaggtcaagttc1140

aactggtacg tggacggcgtggaggtgcat aatgccaagacaaagccgcggggaggagcag1200

tacaacagca cgtaccgtgtggtcagcgtcctcaccgtcc tgcaccaggactggctgaat 1260

ggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacc1320

atctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgg1380

gatgagctga ccaagaaccaggtcagcctgacctgcctag tcaaaggcttctatcccagc 1440

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa ggccacgcct 1500

cccgtgctgg actccgacgg ctccttcttc ctctacagca aggctcaccgt ggacaagagc 1560

aggtggcagc aggggaacgtcttctcatgc tccgtgatgc atgaggctct gcacaaccac 1620

tacacgcagaagagcctctccctgtctccgggtaaa ggtaaa

CN1304427C Angiogenesis inhibiting fusion protein and its use

1.The six fusion proteins obtained from the fusion of the fragments of FLT-1 and KDR and the immunoglobulin Fc fragments have the function of blocking the biological function of vascular endothelial growth factor and inhibiting angiogenesis, and are characterized by the following structures:

a. FP1, a protein fused by the second immunoglobulin-like region of FLT-1 and the third immunoglobulin-like region of KDR: FLTd2-KDRd3-Fc;

b. FP2, a protein fused by the first immunoglobulin-like region of KDR, the second immunoglobulin-like region of FLT-1 and the third immunoglobulin-like region of KDR: KDRd1-FLTd2-KDRd3-Fc;

c. FP3, a protein fused by the second immunoglobulin-like region of FLT-1 and the 3-4 immunoglobulin-like region of KDR: FLTd2-KDRd3, 4-Fc;

d. FP4, a protein fused from the second immunoglobulin-like region of FLT-1, the third immunoglobulin-like region of KDR and the fourth immunoglobulin-like region of FLT-1: FLTd2-KDRd3-FLTd4- Fc;

e. FP5, a protein fused from the second immunoglobulin-like region of FLT-1 and the third 3-5 immunoglobulin-like region of KDR: FLTd2-KDRd3, 4, 5-Fc; or

f. FP6, a protein fused from the second immunoglobulin-like region of FLT-1, the third immunoglobulin-like region of KDR and the 4-5 immunoglobulin-like region of FLT-1: FLTd2-KDRd3- FLTd4,5-Fc.

2.The fusion protein of claim 1, wherein the immunoglobulin FC fragment is selected from human immunoglobulin FC or animal immunoglobulin FC.

3.The fusion protein of claim 1, wherein the immunoglobulin FC fragment is selected from IgG, IgM, IgA or FC fragments of subtypes IgG1, IgG2, IgG3, IgG4.

4.The fusion protein of claim 1, wherein the immunoglobulin FC fragment is a full-length FC or a partial FC sequence selected from CH2 fragments, CH3 fragments, and stranded region fragments.

5.DNA encoding the fusion protein of claim 1.

6.A vector containing the DNA sequence of claim 5, which is selected from plasmids, viruses or DNA fragments.

7.Cells containing the vector of claim 6, which can be used for the expression of the corresponding fusion protein of claim 1, which are selected from eukaryotic cells or prokaryotic cells.

8.Use of the fusion protein of claim 1 in the preparation of a drug that blocks the biological action of vascular endothelial cell growth factor and inhibits angiogenesis.

9.A pharmaceutical composition containing the fusion protein of claim 1.

10.The pharmaceutical composition of claim 9 is an injection, and the active ingredient is fusion protein FP3.

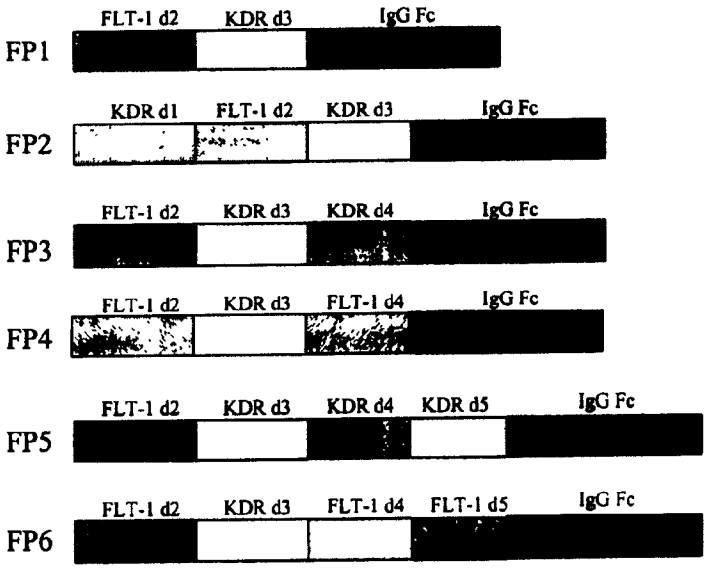


图 1

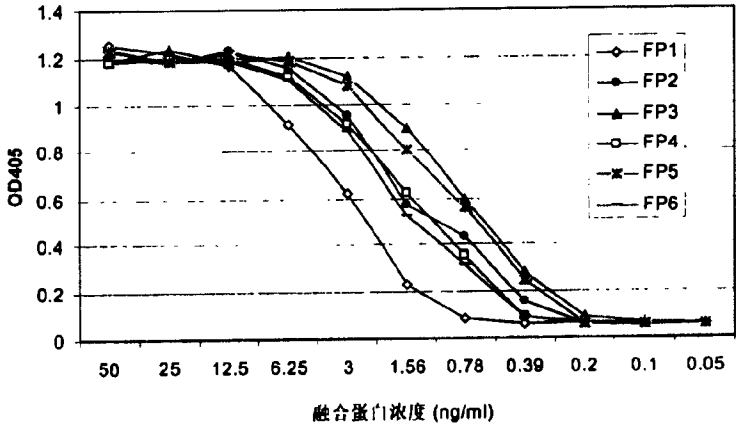


图 2

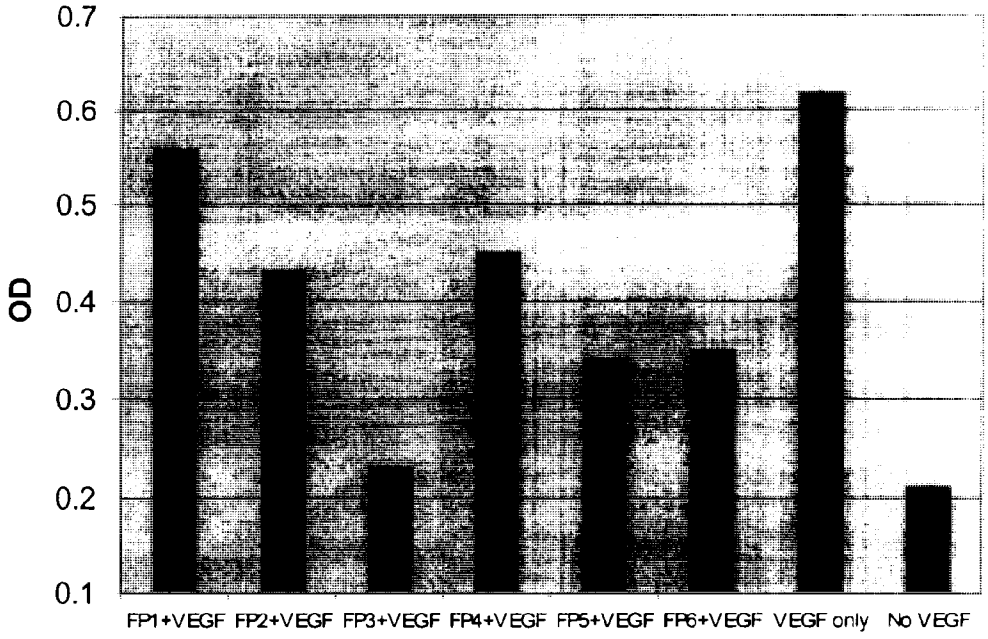


图 3

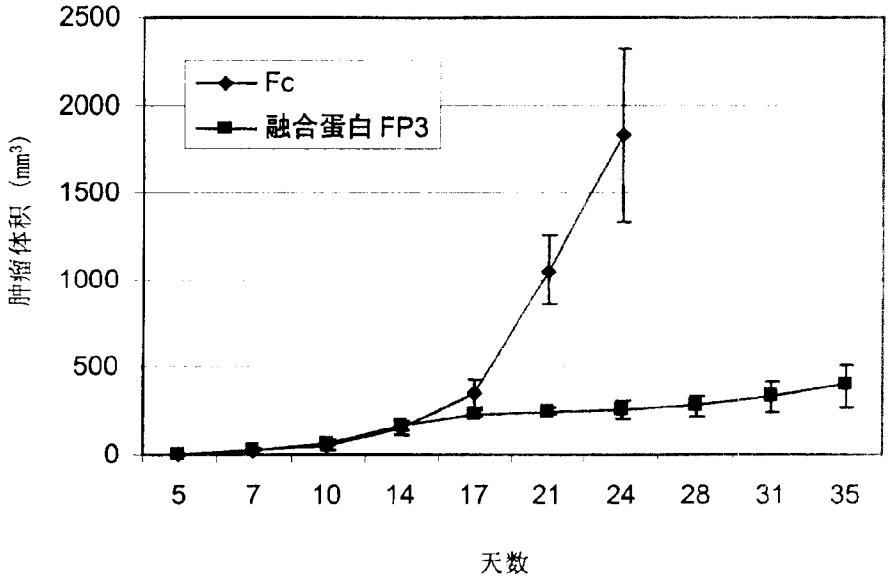


图 4

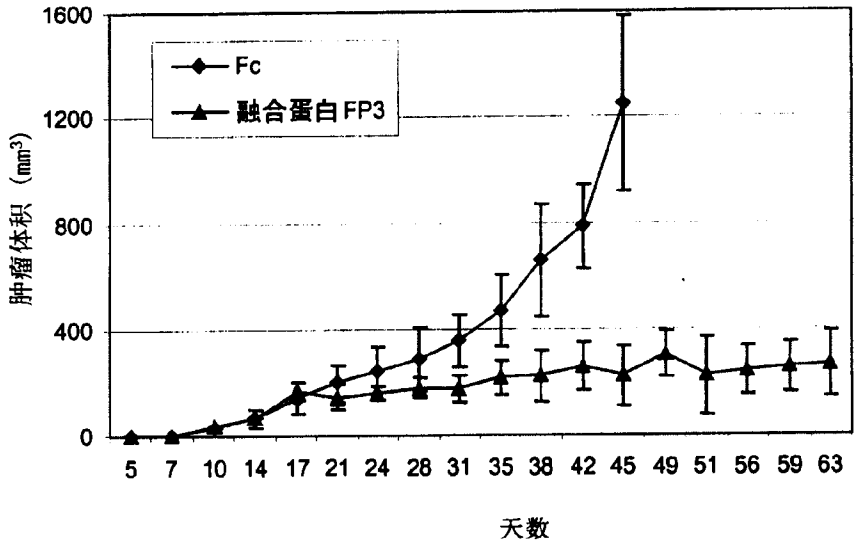


图 5

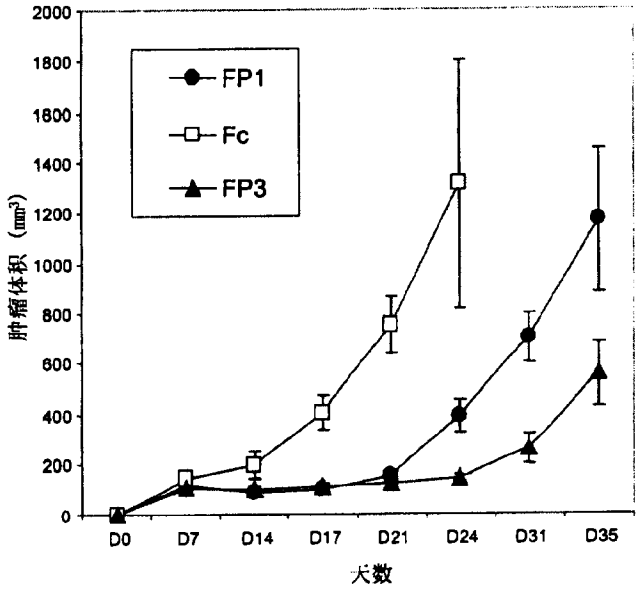


图 6

[19] 中华人民共和国国家知识产权局

[12] 发明专利说明书

专利号 ZL 200510073595.4

[51] Int. Cl.
C07K 19/00 (2006.01)
C12N 15/62 (2006.01)
C12N 15/63 (2006.01)
C12N 15/79 (2006.01)
A61K 39/395 (2006.01)
A61P 35/00 (2006.01)

[45] 授权公告日 2007 年 3 月 14 日

[11] 授权公告号 CN 1304427C

[22] 申请日 2005.6.6

[21] 申请号 200510073595.4

[30] 优先权

[32] 2004.6.8 [33] CN [31] 200410044965.7

[73] 专利权人 成都康弘生物科技有限公司

地址 610036 四川省成都市金牛区蜀西路 36 号

[72] 发明人 刘 征

[56] 参考文献

US 6100071 A 2000.8.8

CN1358197A 2002.7.10

US 6383486 A 2002.5.7

US 6057428 A 2000.5.2

审查员 杨振宇

[74] 专利代理机构 北京华科联合专利事务所

代理人 王 为 涂荣昌

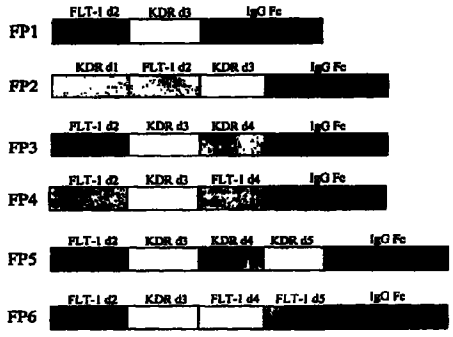
权利要求书 2 页 说明书 15 页 附图 3 页

[54] 发明名称

抑制血管新生的融合蛋白质及其用途

[57] 摘要

本发明涉及一种抑制血管新生的融合蛋白质及其用途，本发明的融合蛋白由 FLT－1 和 KDR 的片断以及免疫球蛋白 Fc 片断经融合得到的，称为 FP1、FP2、FP3、FP4、FP5、FP6，其中 FLT－1 和 KDR 免疫球蛋白样区域的氨基酸 FLT－1 D2、FLT－1 D4、KDR，D1、KDR D3、KDR D4、FP3 序列见序列表 1－6，FP3 的编码 DNA 序列见序列表 7。



1. 由 FLT-1 和 KDR 的片断以及免疫球蛋白 Fc 片断经融合得到的六种具有阻断血管内皮细胞生长因子生物作用、抑制血管新生的融合蛋白，其特征在于，具有以下结构：

   a. FP1，由 FLT-1 的第 2 免疫球蛋白样区域和 KDR 的第 3 免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3-Fc；

   b. FP2，由 KDR 的第 1 免疫球蛋白样区域, FLT-1 的第 2 免疫球蛋白样区域和 KDR 的第 3 免疫球蛋白样区域融合而成的蛋白：KDRd1-FLTd2-KDRd3-Fc；

   c. FP3，由 FLT-1 的第 2 免疫球蛋白样区域和 KDR 的第 3-4 免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3,4-Fc;

   d. FP4，由 FLT-1 的第 2 免疫球蛋白样区域, KDR 的第 3 免疫球蛋白样区域和 FLT-1 的第 4 免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3-FLTd4-Fc；

   e. FP5，由 FLT-1 的第 2 免疫球蛋白样区域和 KDR 的第 3-5 免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3,4,5-Fc；或

   f. FP6，由 FLT-1 的第 2 免疫球蛋白样区域, KDR 的第 3 免疫球蛋白样区域和 FLT-1 的第 4-5 免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3-FLTd4,5-Fc。

2. 权利要求 1 的融合蛋白，其特征在于，其中的免疫球蛋白 FC 片段选自人免疫球蛋白 FC 或动物的免疫球蛋白 FC。

3. 权利要求 1 的融合蛋白，其特征在于，其中的免疫球蛋白 FC 片段选自 IgG、IgM、IgA 或亚型 IgG1、lgG2、IgG3、lgG4 的 FC 片段。

4. 权利要求 1 的融合蛋白，其特征在于，其中的免疫球蛋白 FC 片段是 FC 全长或是部分 FC 序列，选自 CH2 片断、CH3 片断、绞合区域片段。

5. 编码权利要求 1 的融合蛋白的 DNA。

6. 含有权利要求 5 中所述 DNA 序列的载体，这些载体选自质粒，病毒或 DNA 片断。

7. 含有权利要求 6 中所述载体的细胞，这些细胞可用于相应的权利要求 1 中的融合蛋白质的表达，它们选自真核细胞或原核细胞。

8. 权利要求 1 的融合蛋白在制备阻断血管内皮细胞生长因子生物作用、抑制血管新生的药物中的应用。

9. 含有权利要求 1 的融合蛋白的药物组合物。

10. 权利要求 9 的药物组合物，是注射剂，其活性成分是融合蛋白 FP3。

抑制血管新生的融合蛋白质及其用途

技术领域

本发明涉及一系列能有效抑制血管新生(angiogenesis)的基因重组蛋白质。血管新生是指从已经存在的血管生长出新的血管的过程。成人体内的血管绝大多数处于静止状态，血管新生只见于少数病理或生理状态，例如肿瘤，糖尿病人的病变视网目，关节炎，贫血器官，增生期子宫内膜等。在肿瘤的发生过程中，血管新生对肿瘤的快速生长起到关键的作用 (Hanahan and Folkman: Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis。Cell。1996，86:353-364)。动物肿瘤模型的研究和人体临床试验已经证明，抑制肿瘤内新生血管的形成可以有效地阻止肿瘤的生长和发展，从而延长病人的生命

血管新生受到多种生物活性物质的调节和控制。主导血管新生过程的主要细胞是构成血管壁最内层的血管内皮细胞。多种生长因子能与血管内皮细胞表面相应的受体结合，经细胞内的信号传递系统调节血管内皮细胞的活动，从而调控血管的新生。

背景技术

在各种生长因子中，血管内皮细胞生长因子(Vascular endothelial cell growth factor，VEGF) 是调节血管新生的最重要的因子(Ferrara: VEGF and the quest for tumor angiogenesis factor。Nat。Rev。Cancer，2002，10: 795-803。Ferrara: Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications。Semin。Oncol。2002，29 (6 sumppl): 10-14)。血管内皮细胞生长因子可由多种细胞分泌，但它在肿瘤细胞常常过量表达。内皮细胞生长因子通过与相应的受体结合而起作用。与 VEGF 相结合的受体主要有两种: FLT-1 和 KDR。在分子结构上，这两种受体均由三个不同的功能区组成。第一个功能区是位于细胞之外的细胞外部分。它由七个免疫球蛋白样区域(d1-d7)组成。这一部分对 VEGF 具有特异亲和性，是 VEGF 与受体相结合的关键部位。第二个功能区是由疏水性氨基酸组成的跨细胞膜部分。第三个功能区是细胞内部分，其中包括酪氨酸激酶基团。在受体被 VEGF 激活后，酪氨酸激酶基团发生磷酸化，

从而启动细胞内部的信号传递系统，最终造成内皮细胞的功能变化，导致血管新生。

FLT-1 和 KDR 主要分布于血管内皮细胞。因此，VEGF 对于血管内皮细胞具有高度专一性的调节作用。VEGF 具有促进内皮细胞分裂，引导内皮细胞迁移，抑制细胞调亡，和诱导血管形态发生等等功能，是血管新生的高效诱导剂。

在肿瘤组织，VEGF 的表达水平比正常组织为高。另外，肿瘤的快速生长常常导致肿瘤内部的缺氧。而氧分压的降低又导致 VEGF 表达的增高。因此，VEGF 是导致肿瘤中血管新生的关键因子。许多动物试验表明，阻断 VEGF 与其受体的结合能有效地抑制肿瘤中血管的新生，从而阻止肿瘤的生长。在其他与血管新生相关的疾病中，例如糖尿病的视网目病变和关节炎的关节病变等等，VEGF 也与这些疾病的发展有密切的关系。

鉴于 VEGF 在肿瘤及其他疾病中所起到的关键作用，能特异性地阻断 VEGF 的蛋白质或化合物将具有治疗这些疾病的可能。例如，研究证明抗 VEGF 的中和抗体能有效的抑制肿瘤的生长(Jain: Tumor angiogenesis and accessibility: role of vascular endothelial growth factor。Semin。Oncol。2002，29 (6 suppl): 3-9)。因此，寻找新的更为有效的 VEGF 阻断剂在临床上具有重大的意义。由于 FLT-1 和 KDR 对 VEGF 有天然的亲和性，已有研究探讨可溶性 FLT-1(FLT-1 的细胞外部分)或可溶性 KDR(KDR 的细胞外部分)的抗血管新生作用。可溶性 FLT-1 可以在体外有效的抑制血管内皮细胞的增生，但其在血清中的半衰期很短，不能达到有效的血清浓度。可溶性 KDR 在体外也具有抑制血管内皮细胞的增生的作用，但在动物试验中其抗肿瘤效果不佳。FLT-1 的第三免疫球蛋白样区域的部分碱性氨基酸是导致 FLT-1 在体内不稳定的主要原因，因此用 KDR 的部分氨基酸取代这些碱性氨基酸可以增加 FLT-1 的稳定性。

美国专利 6100071、5952199、6383486 描述了几种用 KDR 的部分片段和 FLT-1 部分片段融合的蛋白，但由于其不稳定，副作用大，没有进一步开发。

发明内容

本发明涉及用基因工程技术构建和生产能阻断 VEGF 的蛋白质药物的方法以及这类药物在疾病治疗上的应用。

本发明提供一种由 FLT-1 和 KDR 的片断以及免疫球蛋白 Fc 片断经融合得

到的六种具有阻断血管内皮细胞生长因子生物作用，抑制血管新生的融合蛋白，简称为 FP1、FP2、FP3、FP4、FP5、FP6，具有以下结构：

a. FP1 由 FLT-1 的第 2 免疫球蛋白样区域和 KDR 的第 3 免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3-Fc；

b. FP2 由 KDR 的第 1 免疫球蛋白样区域, FLT-1 的第 2 免疫球蛋白样区域和 KDR 的第 3 免疫球蛋白样区域融合而成的蛋白：KDRd1-FLTd2-KDRd3-Fc；

c. FP3 由 FLT-1 的第 2 免疫球蛋白样区域和 KDR 的第 3-4 免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3,4-Fc;

d. FP4 由 FLT-1 的第 2 免疫球蛋白样区域, KDR 的第 3 免疫球蛋白样区域和 FLT-1 的第 4 免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3-FLTd4-Fc；

e. FP5 由 FLT-1 的第 2 免疫球蛋白样区域和 KDR 的第 3-5 免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3,4,5-Fc；

f. FP6 由 FLT-1 的第 2 免疫球蛋白样区域, KDR 的第 3 免疫球蛋白样区域和 FLT-1 的第 4-5 免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3-FLTd4,5-Fc。

以上 FLT-1 和 KDR 免疫球蛋白样区域的氨基酸 FLT-1 D2、FLT-1 D4、KDR, D1、KDR D3、KDR D4、FP3 序列见序列表 1-6，FP3 的编码 DNA 序列见序列表 7。

其中 FLT 代表 FLT-1 序列，KDR 代表 KDR 序列，d 是 FLT-1 或 KDR 的免疫球蛋白样区域，也可用大写 D 表示(domain)，Fc 为人免疫球蛋白 Fc 序列。

其中的免疫球蛋白 FC 片段选自是人免疫球蛋白 FC 或动物的免疫球蛋白 FC。如：IgG，IgM， IgA 或亚型 IgG1， IgG2， IgG3， IgG4。其中的免疫球蛋白 FC 片段是 FC 全长或是部分 FC 序列，选自 CH2 片断，CH3 片断，绞合区域片段，这些序列和片段都是现有技术，在教科书中可以找到。

本发明还提供本发明的融合蛋白在制备阻断血管内皮细胞生长因子生物作用，抑制血管新生的药物中的应用。

这一发明的关键在于根据 FLT-1 和 KDR 的结构设计构建了一系列由不同

的 FLT-1 片段，KDR 片段，和人免疫球蛋白 Fc 相融合而成的融合蛋白质，再用 VEGF 结合试验等方法筛选对 VEGF 具有最大亲和性的融合蛋白质。从而得到最优的 VEGF 阻断剂。融合蛋白质的构建技术基于分子克隆方法，具体实验方法可参考<<分子克隆>>第二版和第三版等实验手册。

根据 FLT-1 和 KDR 分子各区域的氨基酸序列结构，在上列融合蛋白中，FP1 融合蛋白将可以提供与 VEGF 相结合的基本结构，它由 FLT-1 的第二免疫球蛋白区域(FLTd2)序列，KDR 的第三免疫球蛋白区域(KDRd3)序列和 Fc 组成。FP2 融合蛋白中增加了来自 KDR 的第一免疫球蛋白样区域(KDRd1)的氨基酸序列，这些序列可以增加与 VEGF 相结合的位点，从而增加对 VEGF 的亲和力。FP3 和 FP4 融合蛋白中增加了 FLT-1 或 KDR 的第四(FLTd4 或 KDRd4) 免疫球蛋白样区域的序列。FP5 和 FP6 是在 FP1 的基础上增加了 FLT-1 或 KDR 的第四和第五免疫球蛋白样区域(FLT-1d4，5，KDRd4，5)。这些新增的序列将有利于融合蛋白之间的偶联，从而进一步形成有利于和 VEGF 相结合的空间结构，增加与 VEGF 结合的亲和力。

本发明的融合蛋白可通过基因重组技术获得，该技术为常规技术，首先获得编码有上述融合蛋白的 DNA，DNA 的获得可以通过常规技术，如 PCR 合成等方法，在用 PCR 合成后被克隆到载体中。所用载体可以是分子生物学所常用的质粒、病毒或 DNA 片断。各融合蛋白的氨基末断前加上蛋白分泌信号序列以保证蛋白质从细胞中分泌出来。载体序列中包括用于驱动基因表达的启动子，蛋白质翻译起始和终止信号，以及多聚腺苷酸(PolyA)序列。载体中有抗菌素抗性基因以利于质粒在细菌中所繁殖。另外，载体中还包括真核细胞选择性基因用于稳定转染细胞株的选择。

由于 FLT-1 和 KDR 中各个免疫球蛋白样区域的氨基酸序列之间并没绝然的分界，因此各免疫球蛋白样区域的氨基酸序列的长度可以有一定的变化。所以，本发明所涉及的融合蛋白质的氨基酸序列也可以有一定的变化。它们都属于本发明的范围。

在完成上列各种融合蛋白的质粒构建以后，即可用质粒 DNA 转染细胞，表达相应的蛋白质。能够用于表达这些融合蛋白的表达系统有多种，它们包括(但不限于)哺乳动物细胞，细菌，酵母，昆虫细胞，等等（其中哺乳动物细胞和昆

虫细胞为真核细胞，细菌和酵母细胞为原核细胞。从哺乳动物细胞所表达的蛋白质具有糖基修饰。由于本发明的融合蛋白质的氨基酸序列中包括可糖基化的氨基酸，因此，哺乳动物细胞是表达这些蛋白质的最佳细胞。可用于蛋白质大规模表达的哺乳动物细胞有多种，例如 293 细胞，CHO 细胞，SP20 细胞，NS0 细胞，COS 细胞，BHK 细胞，PerC6 细胞，等等。许多其他细胞也可用于这些蛋白质的表达和生产，因此都包括在本发明所能使用的细胞之列。编码多肽的质粒可经转染(transfection)进入细胞。转染细胞的方法有多种，其中包括(但不限于)：电钻孔(electroporation)，脂质体(liposome)介导，钙介导，等等。

除了哺乳动物细胞，其他表达系统也能用于这些多肽的表达，例如细菌，酵母，昆虫细胞，等等。它们也包括在本发明所能使用的细胞之列。这些表达系统的蛋白质产量比哺乳动物细胞为高，但是，所表达的蛋白质缺乏糖基化或者所形成的糖链与哺乳动物细胞不同。

融合蛋白质表达后，可用酶联免疫吸附试验(ELISA)或其他方法测定细胞培养液中融合蛋白质的浓度。由于这些融合蛋白质具有免疫球蛋白 Fc 片断，因此可用蛋白 A 亲和层析法提取所表达的融合蛋白质。

本发明从质粒转染的 293 细胞培养液中获得相应的各种融合蛋白质。然后利用 VEGF 结合试验来比较各种蛋白质对 VEGF 的亲和力。进而，利用 VEGF 诱导下的人血管内皮细胞分裂试验检测和比较各融合蛋白对 VEGF 的阻断作用。实验结果证明本发明所构建的各种融合蛋白质对 VEGF 具有高度的亲和力(见图 2)，而且，它们能有效地阻断 VEGF 对血管内皮细胞刺激，抑制内皮细胞的分裂。进一步的试验发现，FP3 对 VEGF 的阻断效果最好。是阻断 VEGF 最有效的融合蛋白质。

因此，本发明所构建的融合蛋白质对 VEGF 具有阻断作用。其中 FP1 的分子量最小。FP3 对阻断 VEGF 最为有效。这些融合蛋白质都具有抗血管新生的生物学特性，从而具有治疗疾病可能。

由于本发明的各种融合蛋白质的基本作用是阻断 VEGF，因此这些蛋白质可能应用于与血管新生或者 VEGF 相关的疾病。这些疾病可能包括(但不限于)各种肿瘤，视网目血管病变，关节炎，等等。融合蛋白可以作为提取的重组蛋白质注射到病人体内。也可以将融合蛋白 DNA 序列插入到适当的载体中，用基因

治疗或细胞治疗的方法在病人体内表达。所以，本发明所涉及的融合蛋白质的使用方法有多种形色，不仅包括蛋白质本身，也包括编码融合蛋白的 DNA。

为了进一步证明融合蛋白质在体内的抗血管作用，本发明试验了 FP3 融合蛋白质在动物体内的抗肿瘤作用。在小鼠 B16F10 黑色素瘤和异种移植(Xenograft)模型人 PC-3 前列腺癌，融合蛋白质非常有效地抑制了肿瘤的生长，延长了个体的生命。因此，本发明所构建的融合蛋白质具有高效的抗癌能力。

本发明还包括含有本发明融合蛋白的药物组合物，组合物中可以含有药物可接受的载体。组合物可以以任何形式的药物制剂形式存在，优选的是注射剂，最优选的是冷冻干燥注射剂，该药物制剂形式药物组合物，可以按照制剂学常规技术制备，包括将药物活性成分，本发明的融合蛋白与药物载体混合，按照制剂学常规技术制成所需要的剂型。

本发明的融合蛋白，与现有技术相比，优点明显，具有稳定性好，产率高，副作用小的特点，本发明特别优选的融合蛋白是 FP3，是经过大量筛选得到的，经与本发明的其他融合蛋白以及现有技术中其他类似的融合蛋白进行比较实验研究，发现，该融合蛋白具有特别优良的在动物体内的抗肿瘤作用。在小鼠 B16F10 黑色素瘤和异种移植(Xenograft)模型人 PC-3 前列腺癌，FP3 融合蛋白非常有效地抑制了肿瘤的生长，延长了个体的生命，实验证明，FP3 比其他融合蛋白更有效，副作用更小，制成制剂后稳定性最好。在本发明融合蛋白质中最具抗癌应用价值。

附图说明

图 1 展示了六种融合蛋白的结构组成。它们由不同的 FLT-1 和 KDR 多肽片断以及免疫球蛋白 Fc 片断用基因工程构建而成。

图 2 显示六种融合蛋白与 VEGF 结合的试验结果。OD 读数代表融合蛋白质与 VEGF 的结合信号。结果显示它们与 VEGF 具有很强的结合能力，尤其以 FP3 的结合能力最强。

图 3 融合蛋白有效地抑制人血管内皮细胞的在体外的分裂。

图 4 显示融合蛋白质 FP3 有效地抑制小鼠 B16F10 黑色素瘤在体内的生长。

图 5 显示融合蛋白质有效地抑制人 PC-3 前列腺癌在小鼠体内的生长。

图 6 融合多肽 FP1 与 FP3 有效地抑制小鼠肿瘤的生长的比较研究。

具体实施方式

以下实例对本发明所涉及的融合蛋白构建，试验和应用作了详细说明。但是本发明的内容及用途并不限制于实例的范围。

实施例 1: 融合蛋白质及其质粒的构建。

除了免疫球蛋白 Fc 片断，构建本发明中各种融合蛋白的原始序列来自 FLT-1 和 KDR 相应的 cDNA。由于 FLT-1 和 KDR 的表达主要见于血管内皮细胞，故本发明用 RNA 提纯药盒(QIAGEN 公司)从人脐带静脉血管内皮细胞(HUVEC)提取了总 RNA。然后用逆转录酶从 RNA 合成 cDNA。再用不同的引物利用聚合酶链反应(PCR)扩增获得所需要的 FLT-1 和 KDR 片断。最后用 PCR 将来自 FLT-1，KDR，和人免疫球蛋白 Fc (IgG1 Fc) 序列相融合，从而构建成不同融合蛋白质的 DNA 序列。六种融合蛋白的结构见图 1。

FP3 基因的构建：

用 EGM-2 培养基（Clonetics）在 T－175 培养瓶中培养人脐带静脉血管内皮细胞(HUVEC 细胞)（Clonetics)。收集大约 1×10e7 个细胞，利用 Qiagen 公司的 RNA 提取试剂盒提取细胞总 RNA，并用 Invitrogen cDNA 试剂盒合成 cDNA，-80℃冻存备用。采用下列特异引物从 HUVEC cDNA 中扩增到 FLT-1 and KDR 基因片段。

用人 IgG1 Fc 特异引物从来源自淋巴结(BD Clontech)的 cDNA 中扩增到 IgG1 Fc 基因片段。

Primers（引物）：

FLT-1 D2 forward: cctttcgtagagatgtacagtga

FLT-1D2 reverse: tatgattgtattggtttgtccat

KDR D3-4 forward: gatgtggttctgagtccgtctca

KDR D3-4 reverse: cggtgggacatacacaaccaga

Human IgG1 Fc forward: gacaaaactcacacatgcccact

Human IgG1 Fc reverse: tcatttacccggagacagggagag

在变性 95℃，30 分，退火 56℃，45 秒，延伸 72℃，2 分的条件下，进行 PCR 扩增，30 个循环，获得 FLT-1 和 KDR IgG 样结构域的 PCR 产物 及人 IgG1 Fc 段 PCR 产物。用 TA cloning 试剂盒，把 PCR 产物克隆入 pCR2.1 质粒，并转染 E.coli，选取白色菌落，加入 LB 培养基，培养过夜。Qiangen 质粒提取试剂盒提取质粒后酶切，及测序鉴定。

采用拼接 PCR(sewing PCR)方法，把 FLT-1、KDR 和 IgG Fc cDNA 连接在一起，在引物中设计有 EcoRI 的酶切位点，用 EcoRI 酶切后，Qiangen 纯化试剂盒纯化 DNA 片段并插入 pcDNA3.1 质粒，重组质粒转染 E.coli，选取阳性菌落，加入 LB 培养基，培养过夜。Qiagen 质粒提取试剂盒提取质粒后酶切，及测序鉴定。已获证实的质粒再转染 293 或 CHO 细胞获得稳定表达 FP3 的细胞系。FP3 的具体氨基酸序列见序列表 6，

实施例 2: 融合蛋白质在细胞中的表达。

本发明的组成部分之一是在细胞中表达所构建的融合蛋白质。在完成各质粒的构建以后，用质粒 DNA 提纯药盒(QIAGEN 公司)提取了高纯度质粒 DNA。然后，利用 FUGEN6 质粒转染药盒(ROCHE 公司)将质粒 DNA 导入 293 细胞中。按所需蛋白质量的多少，采用了两种不同的质粒转染的方法表达融合蛋白。

第一种方法是瞬时转染(Transient transfection)法，用这一方法可以得到小规模量的融合蛋白质。首先用含 10%胎牛血清的 DMEM 完全培养基将 293 细胞在细胞培养皿内培养。当细胞生长至覆盖 60-80%面积时，将质粒 DNA 与 FUGEN6 试剂的复合物加入细胞培养液中。第二天，用无血清 DMEM 培养基换液。再继续培养三天，然后收集上清液。这些上清液中含有从细胞表达的融合蛋白多肽。融合多肽的浓度用 ELISA 法定量确定。用这一方法，本发明表达了上述六种融合多肽。

第二种方法是用稳定转染(Stable transfection)法建立稳定细胞，以表达大量的融合蛋白多肽。所用细胞亦是 293 细胞。质粒的转染方法与上述瞬时转染法相同，但是，在转染第二天，细胞在含有新酶素的 DMEM 完全培养中用有限密度稀释法经行克隆培养。大约 21 天后，挑取新酶素抗性克隆，进行细胞的扩大培养。最后，细胞在转鼓培养瓶中培养生产融合多肽。融合多肽的浓度用 ELISA 法定量确定。

本发明证明可以从质粒经细胞转染表达和生产所构建的融合多肽。

实施例 3: 融合多肽与 VEGF 的结合实验。

本发明用 VEGF 结合试验测定了各融合多肽与 VEGF 的结合能力。在这一试验中，首先将重组 VEGF(Chemicom 公司)蛋白质包被在 96 孔 ELISA 板上。然后用百分之五的牛奶粉溶液阻断非特异性蛋白结合位点。再向各孔加入含有不同浓度的各种融合蛋白质，在 37 度培养两个小时。清洗以后，加入兔抗人 Ig 抗体-HRP。最终用过氧化物酶底物显色。用 ELISA 阅读仪测量 96 孔板各孔 OD 值。高 OD 值代表融合蛋白质与 VEGF 的结合信号。

如图 2 所示，本发明所构建和表达的六种融合蛋白都具有与 VEGF 结合的能力。在每毫升 1 毫微克的浓度，便能检测到它们与 VEGF 的结合信号。因此，这六种融合蛋白都与 VEGF 具有很高的亲和力。但是，比较而言，FP3 与 VEGF 的结合能力最大，是最强大的 VEGF 阻断剂。它的半数最大结合浓度(Half maximal binding concentration)比 FP1 低约 5 倍。FP5 与 VEGF 的结合能力比 FP3 稍弱。这一结果说明 KDR 的第四免疫球蛋白样区域的氨基酸序列可增进融合蛋白对 VEGF 的阻断能力。但是，在融合蛋白中进一步增加 KDR 的序列，例如第五免疫球蛋白样区域，并不进一步增加对 VEGF 的抑制能力。其他三个融合蛋白质的抑制能力比 FP3 和 FP5 为弱，但比 FP1 强。

实施例 4: 融合蛋白有效地抑制人血管内皮细胞的在体外的分裂。

本发明另一关键实例是证明了所构建的融合蛋白能有效地阻断 VEGF 所诱导的血管内皮细胞的分裂。在这一实验中，将脐带静脉血管内皮细胞(HUVEC 细胞，CLONETICS 公司)接种于 96 孔细胞培养板。细胞培养液为 EBM 基本培养基(CLONETICS 公司)，含 2%胎牛血清和 15ng/ml VEGF。在实验组的细胞培养液中，加入含不同浓度融合蛋白的 293 细胞上清液。在阴性对照组的细胞培养液中，加入未经质粒转染的 293 细胞上清液(不含融合蛋白)。不同处理的 HUVEC 细胞在 37 度继续培养。三天后，细胞计数确定各孔中的 HUVEC 密度。

如图 3 所示，六种蛋白质都能有效的阻断 VEGF 对其受体的刺激作用。其中 FP3 对 HUVEC 生长抑制作用最明显。

HUVEC 细胞分裂试验显示，本发明所构建的六种融合蛋白质钧能抑制血管内皮细胞的分裂。鉴于在这个实验中 HUVEC 细胞的分裂由 VEGF 刺激造成，

因此，这六种蛋白质都能有效的阻断 VEGF 对其受体的刺激作用。它们都有抑制血管新生的功能。在这六种蛋白质中，以 FP3 对 HUVEC 细胞分裂的阻抑作用最强，它们的半数抑制浓度 (IC50) 在 3 ng/ml 左右。FP1 的 IC50 为 12 ng/ml 左右。FP2，FP4，FP5，和 FP6 的 IC50 在 5-8 ng/ml。

实施例 5: 融合多肽能有效地抑制小鼠肿瘤的生长。

作为 VEGF 阻断剂，本发明所构建的融合蛋白质的应用之一是用于肿瘤的治疗。鉴于 FP3 融合蛋白对 VEGF 有高效的阻断作用，本发明选择 FP3 进行了动物体内抗肿瘤效果的试验。

本发明所试验的小鼠肿瘤模型是 B16F10 黑色素瘤。这是一种快速生长的恶性肿瘤。在这个试验中，先将 B16F10 细胞注入小鼠背部皮下。经尾静脉注射提纯的融合蛋白质。注射剂量为每小鼠每次 400 微克(小鼠平均体重约 22 克)，每周两次。对照组注射同剂量的提纯人免疫球蛋白 Fc。图 4 显示肿瘤的生长曲线。融合蛋白非常有效抑制了该黑色素瘤的生长($P<0.01$)。

让人类肿瘤细胞在裸鼠体内生长的异种移植(Xenograft)模型是与人体肿瘤最为接近的动物肿瘤模型。裸鼠缺乏免疫排斥能力，因此，许多来自人的肿瘤细胞系能在裸鼠体内生长，形成肿瘤。本发明试验了融合蛋白 FP3 对人前列腺癌 PC-3 细胞在裸鼠体内生长的抑制作用。在这一模型，首先将 PC-3 细胞注入裸鼠背部皮下，从尾静脉注射提纯的融合蛋白。每个小鼠每次 400 微克，每周两次。对照组注射相同剂量的人免疫球蛋白 Fc。实验结果如图 5 所示。在对照组，肿瘤细胞接种后 45 天，肿瘤已长至大于 1000 立方毫米。而在用融合蛋白质注射的动物，融合蛋白几乎完全抑制了 PC-3 肿瘤的生长($P<0.01$)，对肿瘤具有非常显著的治疗作用。

实施例 6: 融合多肽 FP1 与 FP3 有效地抑制小鼠肿瘤的生长的比较研究。

为更好地说明 FP3 具有较好的肿瘤抑制作用，选用了 FP1 同 FP3 做抑制肿瘤生长的比较试验。选用 10 只生长良好的裸鼠，每只背部注射鼠神经胶质瘤 C6 细胞 1×10^5，0.05ml，再尾静脉分别注射提纯的融合蛋白 FP1 和 FP3，2.5mg/kg，每周两次，到 31 天。对照组注射相同剂量的人免疫球蛋白 Fc。实验结果如图 6 所示。FP1 和 FP3 对肿瘤具有非常显著的治疗作用，到第 35 天，FP1 组肿瘤体积是 1167.3，而 FP3 组是 557.6，对照组再第 24 天已到 1312.3。所以，FP3 的作

用更明显（P<0.05.）。结果如图 6 所示。

综上所述，本发明所构建的融合蛋白质对 VEGF 具有高度的亲和性，能在体外抑制血管内皮细胞的增生，并能在体内非常显著抑制肿瘤的生长。鉴于血管新生为所有肿瘤增生之必需，本发明所涉及的融合蛋白质可用于多种肿瘤的治疗。

序 列 表

<110>成都康弘科技实业（集团）有限公司
<120>抑制血管新生的融合蛋白质及其用途
<160>7
<210>1
<211>93
<212>PRT
<213>人工序列
<400>1

```
Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu
1               5                  10                  15                  20
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro
                25                  30                  35                  40
Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile
                45                  50                  55                  60
Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly
                65                  70                  75                  80
His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr
                85                  90          93
```

<210>2
<211>96
<212>PRT
<213>人工序列
<400>2

```
Phe Ile Thr Val Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
1               5                  10                  15                  20
Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val Trp Leu Lys Asp
                25                  30                  35                  40
Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu Thr Arg Gly Tyr Ser Leu Ile Ile
                45                  50                  55                  60
Lys Asp Val Thr Glu Glu Asp Ala Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser
                65                  70                  75                  80
Asn Val Phe Lys Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro
                85                  90                  95  96
```

<210>3
<211>86
<212>PRT
<213>人工序列
<400>3

```
Pro Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr Leu Gln Ile
1               5                  10                  15                  20
Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro Asn Asn Gln Ser Gly Ser Glu
                25                  30                  35                  40
Gln Arg Val Glu Val Thr Glu Cys Ser Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro
                45                  50                  55                  60
Lys Val Ile Gly Asn Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala
                65                  70                  75                  80
Ser Val Ile Tyr Val Tyr
                85  86
```

<210>4
<211>102
<212>PRT
<213>人工序列
<400>4

```
Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val Leu Asn
```

1 5 10 15 20
Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser
25 30 35 40
Lys His Gln His Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met
45 50 55 60
Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr
65 70 75 80
Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg Val His
85 90 95 100
Glu Lys
102

<210>5
<211>92
<212>PRT
<213>人工序列
<400>5
Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val
1 5 10 15 20
Arg Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly
25 30 35 40
Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr Ile Met Glu Val
45 50 55 60
Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro Ile Ser Lys Glu Lys
65 70 75 80
Gln Ser His Val Val Ser Leu Val Val Tyr Val Pro
85 90 92

<210>6
<211>552
<212>PRT
<213>人工序列
<400>6
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu
1 5 10 15 20
Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile
25 30 35 40
Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile
45 50 55 60
Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
65 70 75 80
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu
85 90 95 100
Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln
105 110 115 120
Thr Asn Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile Glu
Leu Ser Val Gly
125 130 135 140
Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn
145 150 155 160
Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg Asp Leu Lys Thr
165 170 175 180
Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg
185 190 195 200
Ser Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser
205 210 215 220
Thr Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu
225 230 235 240

```
Val Glu Ala Thr Val Gly Glu Arg Val Arg leu Pro Ala Lys Tyr Leu Gly Tyr Pro Pro
                245                 250                 255                 260
Pro Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala
                265                 270                 275                 280
Gly His Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile
                285                 290                 295                 300
Leu Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val Val Tyr Val
                305                 310                 315                 320
Pro Pro Gly Pro Gly Asp Lys Thr His Thr Cys Pro Leu Cys Pro Ala Pro Glu Leu Leu
                325                 330                 335                 340
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                345                 350                 355                 360
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
                365                 370                 375                 380
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
                385                 390                 395                 400
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                405                 410                 415                 420
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                425                 430                 435                 440
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
                445                 450                 455                 460
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
                465                 470                 475                 480
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Ala Thr Pro
                485                 490                 495                 500
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                505                 510                 515                 520
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
                525                 530                 535                 540
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                545                 550     552
```

<210>7
<211>1656
<212>DNA
<213>人工序列
<400>7

```
atggtcagct  actgggacac  cggggtcctg  ctgtgcgcgc  tgctcagctg  tctgcttctc  60
acaggatcta  gttccggagg  tagacctttc  gtagagatgt  acagtgaaat  ccccgaaatt  120
atacacatga  ctgaaggaag  ggagctcgtc  attccctgcc  gggttacgtc  acctaacatc  180
actgttactt  taaaaaagtt  tccacttgac  actttgatcc  ctgatggaaa  acgcataatc  240
tgggacagta  gaaagggctt  catcatatca  aatgcaacgt  acaaagaaat  agggcttctg  300
acctgtgaag  caacagtcaa  tgggcatttg  tataagacaa  actatctcac  acatcgacaa  360
accaatacaa  tcatagatgt  ggttctgagt  ccgtctcatg  gaattgaact  atctgttgga  420
gaaaagcttg  tcttaaattg  tacagcaaga  actgaactaa  atgtggggat  tgacttcaac  480
tgggaatacc  cttcttcgaa  gcatcagcat  aagaaacttg  taaaccgaga  cctaaaaacc  540
cagtctggga  gtgagatgaa  gaaatttttg  agcaccttaa  ctatagatgg  tgtaacccgg  600
agtgaccaag  gattgtacac  ctgtgcagca  tccagtgggc  tgatgaccaa  gaagaacagc  660
acatttgtca  gggtccatga  aaacctttct  gttgcttttg  gaagtggcat  ggaatctctg  720
gtggaagcca  cggtggggga  gcgtgtcaga  atccctgcga  agtaccttgg  ttacccaccc  780
ccagaaataa  aatggtataa  aaatggaata  ccccttgagt  ccaatcacac  aattaaagcg  840
gggcatgtac  tgacgattat  ggaagtgagt  gaaagagaca  caggaaatta  cactgtcatc  900
cttaccaatc  ccatttcaaa  ggagaagcag  agccatgtgg  tctctctggt  tgtgtatgtc  960
ccaccgggcc  cgggcgacaa  aactcacaca  tgcccactgt  gcccagcacc  tgaactcctg  1020
gggggaccgt  cagtcttcct  cttccccccа  aaacccaagg  acaccctcat  gatctcccgg  1080
acccctgagg  tcacatgcgt  ggtggtggac  gtgagccacg  aagaccctga  ggtcaagttc  1140
```

```
aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1200
tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1260
ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagccccat cgagaaaacc 1320
atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 1380
gatgagctga ccaagaacca ggtcagcctg acctgcctag tcaaaggctt ctatcccagc 1440
gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa ggccacgcct 1500
cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc 1560
aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1620
tacacgcaga agagcctctc cctgtctccg ggtaaa                          1656
```

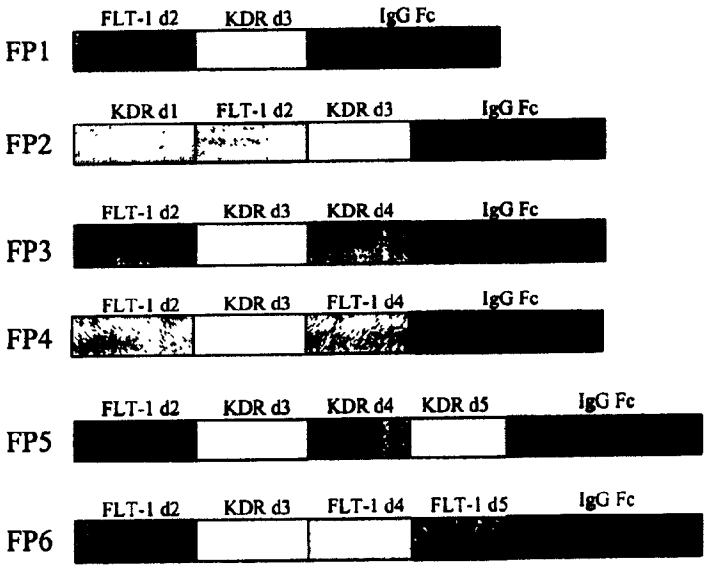


图 1

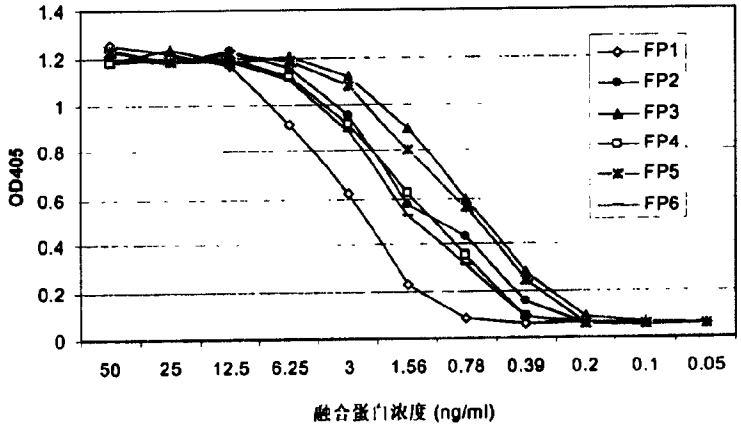


图 2

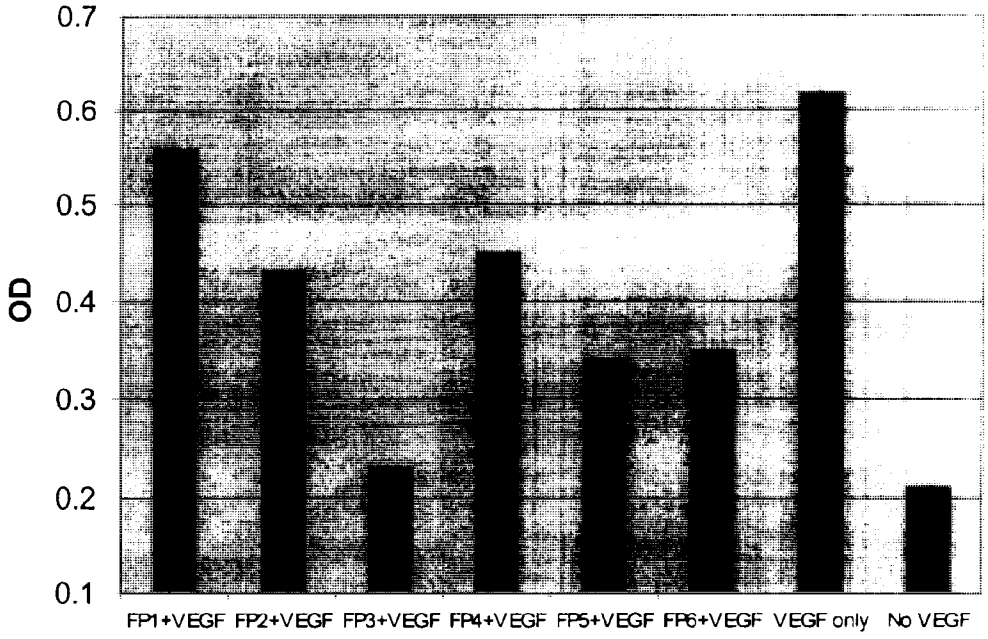


图 3

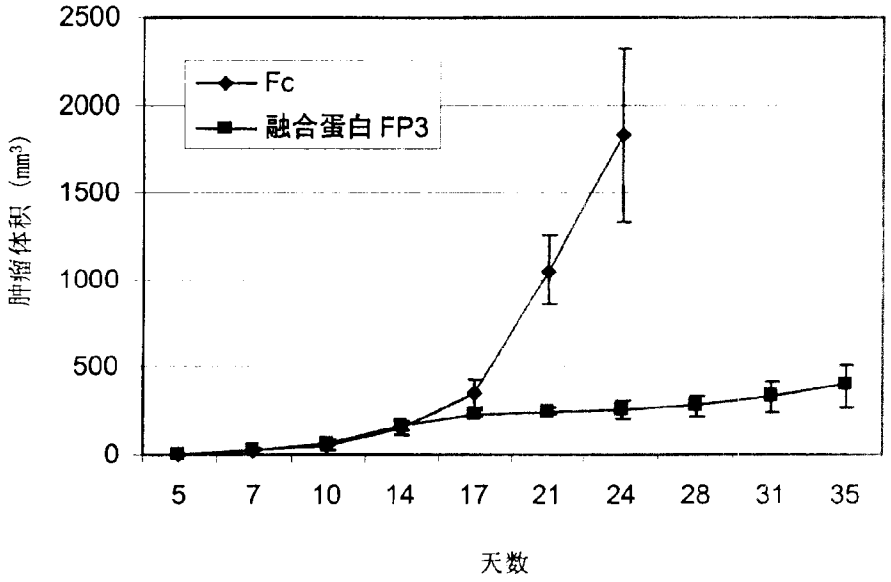


图 4

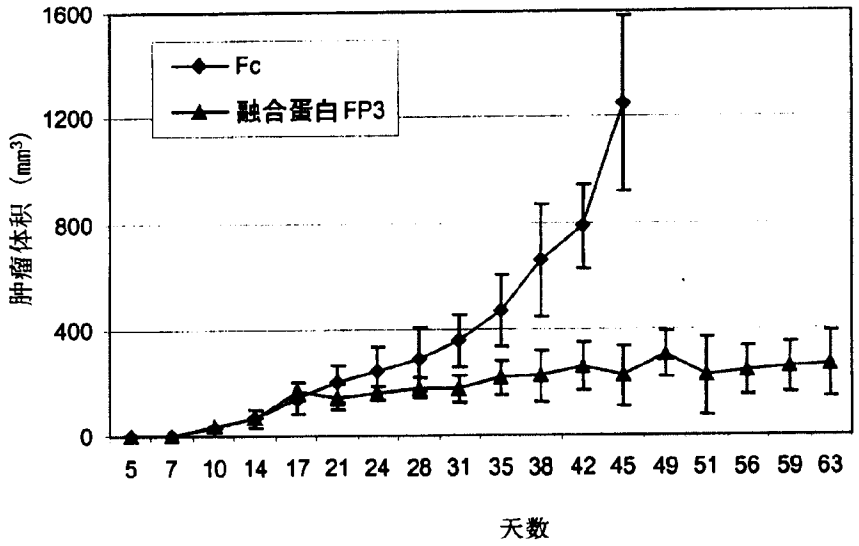


图 5

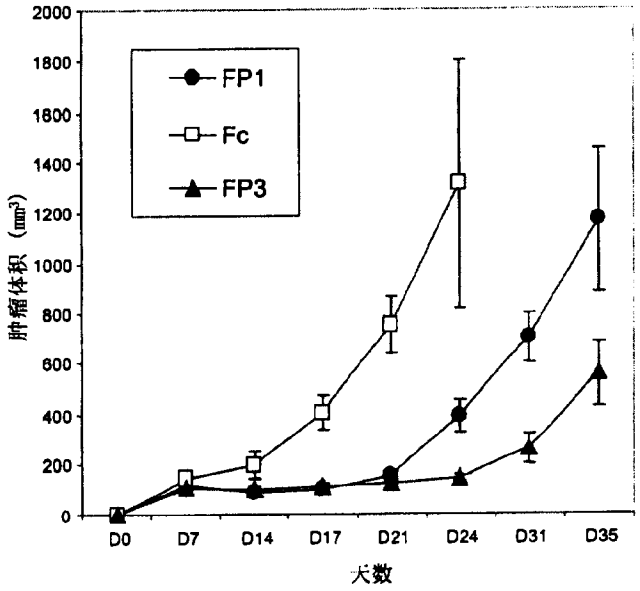


图 6

[19] 中华人民共和国国家知识产权局

[51] Int. Cl.
A61K 39/395 (2006.01)
A61K 38/18 (2006.01)
A61P 27/02 (2006.01)

[12] 发明专利说明书

专利号 ZL 200610066257.2

[45] 授权公告日 2009 年 6 月 24 日

[11] 授权公告号 CN 100502945C

[22] 申请日 2006.3.31

[21] 申请号 200610066257.2

[73] 专利权人 成都康弘生物科技有限公司

地址 610036 四川省成都市金牛区蜀西路 36 号

[72] 发明人 俞德超

[56] 参考文献

WO2005027973A2 2005.3.31

CN1706867A 2005.12.14

CN2005020972A2 2005.3.10

血管内皮生长因子及其受体与眼内新生血管性疾病. 赵世红综述，何守志审校. 眼科研究，第 21 卷第 1 期. 2003

血管内皮生长因子受体的结构与功能. 张曼等. 生物化学与生物物理进展，第 24 卷第 1 期. 1997

审查员 丁 海

[74] 专利代理机构 北京华科联合专利事务所

代理人 王 为

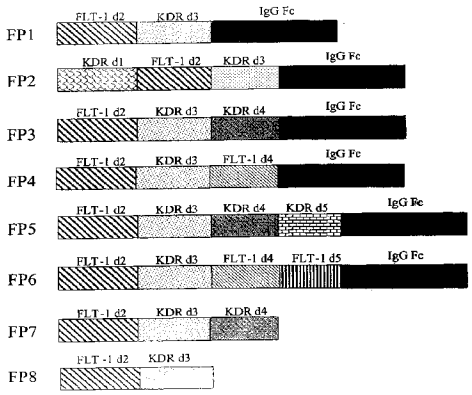
权利要求书 1 页 说明书 13 页 附图 4 页

[54] 发明名称

VEGF 受体融合蛋白在治疗眼睛疾病中的应用

[57] 摘要

本发明涉及 VEGF 受体融合蛋白在治疗眼睛疾病中的应用，所述眼睛疾病包括年龄相关性黄斑变性，糖尿病视网膜病变，糖尿病黄斑瘤腺体以及由新生血管生长而引发的治疗失败如激光凝固体，手术视网膜移植。



1. 一种血管内皮生长因子 VEGF 的受体融合蛋白 FP3 在制备治疗由新生血管生长而引发的眼科疾病的药物中的应用，其中 FP3 的氨基酸序列如序列表中的序列 5 所示。

2. 一种血管内皮生长因子 VEGF 的受体融合蛋白 FP3 在制备治疗由视网膜缺血性萎缩导致新生血管形成，从而引发眼科疾病的药物中的应用，其中 FP3 的氨基酸序列如序列表中的序列 5 所示。

3. 一种血管内皮生长因子 VEGF 的受体融合蛋白 FP3 在制备治疗由脉络膜新生血管生长，从而引发眼科疾病的药物中的应用，其中 FP3 的氨基酸序列如序列表中的序列 5 所示。

4. 根据权利要求 1 所述的应用，所述眼科疾病包括年龄相关黄斑变性，糖尿病视网膜病变，糖尿病性黄斑水肿，视网膜血管阻塞。

5. 如权利要求 1-4 任一项所述的应用，所述药物中还含有药学上可接受的载体。

6. 如权利要求 5 所述的应用，所述药学上可接受的载体选自磷酸盐缓冲液、琥珀酸盐缓冲液、组氨酸缓冲液、甘露醇、海藻糖、聚山梨醇酯、氯化钠、蔗糖、三羟甲基氨基甲烷或乳糖。

7. 如权利要求 1-4 任一项所述的应用，所述融合蛋白能够制成任何适合的药物制剂形式。

8. 如权利要求 7 所述的应用，所述制剂选自冻干制剂或溶液制剂。

9. 如权利要求 1-4 任一项所述的应用，所述药物的给药方式包括玻璃体内注射、静脉给药、腹腔注射、皮下注射或以眼药水滴眼方式给药。

10. 如权利要求 1-4 任一项所述的应用，其中的融合蛋白能够和其他治疗方法结合使用以降低新生血管生长引起的治疗失败机率。

VEGF 受体融合蛋白在治疗眼睛疾病中的应用

技术领域：

本发明涉及 VEGF 受体融合蛋白在治疗因新生血管的生长而引发的各种眼科疾病中的应用。

背景技术：

视网膜血管系统和脉络膜(chorodial) 血管系统是组成视网膜的重要组成部分。创伤或疾病而引起的血管管壁结构或功能的异常改变是导致视力下降或丧失的主要原因。例如，糖尿病视网膜病变（diabetic retinopathy）是由于糖尿病而导致视网膜血管增生，进而造成视网膜脱落。它是造成视力丧失的主要原因。在眼睛受伤后或手术后的恢复过程中也可能造成视网膜内新生血管的生长。这一类视网膜血管增生也是造成视力下降或眼睛失明的重要原因（Nature 438: 932-938, 2005）。

年龄相关的黄斑变性（AMD）是一种由视网膜中心区的细胞或组织退化和血管增生而引起的疾病。可分干性和湿性两种。其中湿性 AMD 是脉络膜新生血管形成的最常见形式，它也是引起眼睛失明的主要原因。

血管内皮生长因子(VEGF)是一种专一性调控新生血管生长的蛋白质(Am. J. Pathol. 167:1451-1459, 2005)。VEGF 刺激内皮细胞分裂增生，进而促进新生血管生长，以提供营养和氧气给组织细胞。很多研究表明一旦眼睛视网膜的感光细胞由于营养不足而开始萎缩（称作“缺血性萎缩”），VEGF 在视网膜内的浓度便开始升高，从而促进新生血管生长。这一过程称为“新生血管的形成”（angiogenesis）。在眼内，新生的血管在形态上与正常血管不同，管腔不规则，管壁多为渗漏。这种高通透性或渗漏的血管的异常增生常导致视网膜上产生疤痕，并进一步可发生脱落从而影响到视力。

许多研究表明湿性 AMD 病人的脉络膜组织中有高水平的 VEGF 表达(Invest. Opthal. Vis. Sci 37 :855－868,1996 ; Microvascular Res. 64:162－169, 2002)。由于 VEGF 表达水平与湿性 AMD 之间的相关性，VEGF 可以被用作诊断 AMD 的一个生化指标（Br. J. Opthalmol. 88:809－815,2004）。

一些 VEGF 抑制剂可以阻断 VEGF 与内皮细胞上 VEGF 受体（flt—1、KDR 等）之间的相互作用，从而阻止由 VEGF 介导的信息传导，抑制由 VEGF 高表达而引起的新生血管的生长，以达到预防和阻止视网膜上出血的目的。这类 VEGF 抑制剂包括 Macugen (pegaptanib sodium), Lucentis, VEGF-Trap, Avastin (bevacizumab) 和 AdPEDF 等。其中 Macugen 和 Avastin 已由美国国家食品和药物管理局（FDA）批准上市。

发明内容：

本发明提供一类抑制 VEGF 的融合蛋白，可用于治疗由于新生血管的生长而引发的各种眼科疾病。所述疾病包括（但不限于）所述眼睛疾病包括年龄相关性黄斑变性，糖尿病视网膜病变，糖尿病黄斑瘤腺体以及由新生血管生长而引发的治疗失败如激光凝固体，手术视网膜移植。在本发明中，所述融合蛋白是指大分子化合物，特别是 VEGF 受体的重组融合蛋白。更具体而言，本发明的 VEGF 抑制剂是中国专利申请“抑制血管新生的融合蛋白质及其用途”（申请号 CN200510073595.4）中描述的 FP_1、FP_2、FP_3、FP_4、FP_5、FP_6和本发明提供的 FP_7 和 FP_8 中的任何一种。其中 FP_1、FP_2、FP_3、FP_4、FP_5、FP_6 的融合蛋白氨基酸序列在上述专利说明书中，FP_7 和 FP_8 的氨基酸序列在本发明序列表中的序列 1 和序列 2。

本发明所述的 VEGF 受体融合蛋白可以通过上述专利说明书中的方法制备，其中 FP_1、FP_2、FP_3、FP_4、FP_5、FP_6 是已知物，FP_7 和 FP_8 是新物质，对于新物质，本发明提供了其优选的制备方法实施例，其制备原理和上述专利说明书相同。

本发明经过重组技术制成的制品经过纯化达到药用纯度，然后根据制剂的需要再将其制备成药物制剂，这些制剂应该特别适合于静脉注射给药，玻璃体内注射给药，腹腔注射，皮下注射，局部眼内给药，其制备工艺可以采用药物制剂的常规制法完成，优选的是溶液制剂或干粉制剂，作为干粉制剂，使用时将干粉溶解使成为溶液。本发明的融合蛋白制剂，需要时可以加入药物可接受的载体，所述载体可以是任何适合本发明的制剂形式的药物载体，优选的选自：磷酸钠（sodium phosphate）、琥珀酸钠（sodium succinate）、组氨酸（histidine）、甘露醇（mannitol）、海藻糖（trehalose dihydrate）、聚山梨醇酯（polysorbate 20）、氯化钠（sodium chloride）、蔗糖（sucrose）、三羟甲基氨基甲烷（trometamol）或乳糖，上述制剂缓冲液（formulation buffer），应含有 pH 缓冲系统如磷酸盐

（phosphate）、柠檬酸盐（citrate）、乙酸盐（acetate）、琥珀酸盐（succinate）、三羟甲基氨基甲烷（trometamol，又名 Tris）或组氨酸（histidine）等中的一种，pH 的范围在 3 至 9；可含有渗透压调节剂如氯化钠（sodium chloride）、葡萄糖（dextrose）等；可含有稳定剂如氨基酸（amino acids）、甘油（glycerol）、环糊精（cyclodextrin）、蔗糖（sucrose）、海藻糖（trehalose dihydrate）等；可含有防腐剂如噻汞撒（thimerosal）、亚硫酸氢钠（sodium bisulfite）、苯基乙醇（benzyl alcohol）等。对于冻干制剂，可含有赋形剂如甘露醇（mannitol）等；对于溶液制剂，可含有表面活性剂如聚山梨醇酯（polysorbate 20 或 80）、十二烷基磺酸钠（SDS）等。融合蛋白的浓度范围在 0.01mg/ml 至 1000mg/ml。其用量根据临床的需要而定。另外制剂中根据需要还可加入防腐剂，稳定剂，助溶剂等辅助成分，可以选自任何适宜的药剂学常规的辅助成分。溶剂选择水或其他等渗溶液，缓冲溶液等。

本发明中，同时还介绍了 VEGF 抑制剂的给药方法。这些抑制剂可以通过多种不同的给药途径给予病人，其中包括（但不限于）静脉给药，玻璃体内注射，也可以通过以点眼药的方式在一定剂型下给予病人以达到治疗眼疾的目的。

本发明意外的发现本发明的融合蛋白形式的 VEGF 抑制剂较现有技术具有优良的眼病治疗作用，而且稳定性好，安全性高，副作用小，效果优良，本发明通过实验数据证明了本发明的有益效果，具体数据见实施例。

附图说明：

图 1：本发明的 8 种融合蛋白的结构示意图

图 2：融合蛋白与 VEGF 结合的亲和力的比较

图 3：融合蛋白对视网膜缺血性萎缩引起的新生血管形成的影响

图 4：融合蛋白对脉络膜新生血管生长的影响

具体实施方式：

以下通过实施例进一步说明本发明，但不作为对本发明的限制。

实施例 1，FP_7的构建

融合蛋白 FP7 是由引物 flt－1 D2(F)、 flt—1 D2（R)、KDR D3（F）和 KDR D3－4（R）(见专利申请书，申请号 CN200510073595.4)从 HUVEC 细胞提取到的 mRNA 作为模板合成的 cDNA 上放大到的 flt—1 和 KDR 基因片段重组而成。具体

的条件是在变性 95° 、30 分钟，退火 56° 、45 秒钟，延伸 72° 、2 分钟的条件下，进行 PCR 扩增，30 个循环，获得 flt—1 和 KDR lgG 样结构域的 PCR 产物。用 TA cloning 试剂盒，把 PCR 产物克隆到 PCR2.1 质粒中，并转染 *E. coli*，选取白色菌落，加入 LB 培养基，培养过夜。用 Qiangen 质粒提取试剂盒提取质粒后酶切及测序鉴定。采用拼接 PCR (Sewing PCR) 方法，把 flt—1 片段、KDR 片段和 IgG 铰链区局部序列的核糖核酸一起连接。在两端引物中设计 EcoR1 酶切位点。PCR 终产物在用 EcoR1 酶切后，经 Qiangen 纯化试剂盒纯化 DNA 片段，并插入 pcDNA3.1 质粒。重组质粒转染 *E. coli*，选取阳性菌落，加入 LB 培养基，培养过夜。Qiangen 质粒提取质粒后酶切，并测序鉴定。已获证实的质粒再转染 CHO 细胞就得稳定表达融合蛋白 FP7 的细胞系。FP_7的具体核甘酸序列见序列表 3。在此融合蛋白 C 末端保留有铰链区（hinge）的部分序列。

实施例 2，FP_8的构建

融合蛋白 FP8 是以 FP7 为模板直接用 PCR 扩增而成，PCR 所用引物是 flt-$1D_2$ （F）和 KDR D3-hing (R)。后者的序列为：5'-aggtgctgggcacagtgggcatgtgtgagttttgtctttttcatggaccctgacaaatg。它包括与 KDR 第三免疫球蛋白样区相互补的序列和人 IgG Fc 绞链区的部分核苷酸序列。PCR 扩增和基因克隆的方法与实例 1 相同。最终将插入了 FP8 的 Pc DNA3.1 质粒转染 CHO 细胞，并获得稳定细胞珠，用于蛋白质的表达。FP8 的氨基酸序列见序列表 2，核苷酸序列见序列表 3。

实施例 3 融合蛋白与 VEGF 结合亲和力的实验

本发明用测定 VEGF 的量来确定各种融合蛋白结合 VEGF 的能力。在这一试验中，将一定量的 VEGF（10PM）加入试管中，然后将经稀释的含有不同量的各种融合蛋白加到含有 VEGF 的试管中，混合好后，在 37° 的培养箱中保存一个小时。一个小时以后，试管中游离的 VEGF 由 R&D 系统公司（R&D systems）提供的检测 VEGF 量的试剂盒——VEGF 检测试剂盒（VEGF assay Kit）测定。测定到的结果经软件的处理而得到如图 2 的结果，图 2 表明，FP_1、FP_3和 FP_7都能有效地与 VEGF 亲和结合，其结合亲和能力可由 IC_{50}表示，分别是 11.2PM、4.3PM 和 4.1 PM。这个实验证明，FP_3和 FP_7在体外与 VEGF 的结合能力相似，且两者都高于 FP_1。

这个试验结果进一步说明了 KDR 的第四免疫球蛋白样区域的氨基酸序列可

增进融合蛋白对 VEGF 的结合能力。

实施例 4 融合蛋白阻止由视网膜缺血性萎缩引起的新生血管的形成的实验结果

将出生七天的幼鼠放在含高氧分压（75%±2%）的培养箱内，并控制温度为 23℃±2℃，日光照明。在此条件下培养几天之后，视网膜中心将无血管新生发生，五天之后，将幼鼠放回到正常氧分压的培养箱中。 由于室内相对较低的氧分压浓度而对幼鼠的视网膜产生低氧条件，从而刺激产生类似于糖尿病视网膜病变和其他由于缺血性萎缩视网膜病变的新生血管反应。

利用这个模型，可以对三种融合蛋白（FP_1、FP_3 和 FP_7）在缺血性萎缩视网膜病变相关的新生血管生成方面的作用做出评估。

将幼鼠放在高氧分压的培养箱中，五天之后带回到正常氧分压室内的培养箱中。幼鼠被分成五个组，每组 10 只，一天之后以 30mg/kg 的量将融合蛋白经腹腔注射幼鼠，每两天注射一次，一共注射 4 次。对照组的幼鼠则被注射含相同量的 Fc 蛋白。治疗期结束后，从各组取小鼠 6 只，心脏注射荧光素 FAM，10 分钟后，幼鼠的视网膜被摘取用来分析新生血管的生成情况。操作时将视网膜放平，在荧光显微镜下观察新生血管及荧光渗漏情况，各组其余四只小鼠的眼睛用石蜡包埋，切片后用 H&E 染色。镜下计数血管内皮细胞核的数目，从而判断融合蛋白对新生血管生长的影响（Investigative Ophthalomogy visual science 43, 1994—2000, 2002）。结果如图 3 所示。接受 Fc 蛋白注射的幼鼠视网膜都表现出严重的病变。在视网膜表面内脉膜上可观察到大量杂乱无章的血管。在经融合蛋白处理过的幼鼠其视网膜上则无明显的血管生长（如图 3）.其中 FP_3 最为有效，FP_7 也较有效，但其效果类似于 FP_1，其主要原因可能是因缺失 Fc 片段而影响它在体内的稳定性。FP_1 与 FP_7 的效果类似。

同时，我们也试验了将这些融合蛋白在通过玻璃体内给药的情况下对新生血管的影响。试验采用同样的动物模型，在动物回到正常氧分压的培养箱内一天后，以每只眼睛 0.5 毫克的量进行玻璃体内注射，每只动物只接受一次治疗。给药之后第七天，动物的视网膜按上述方法收集和处理。融合蛋白对新生血管的影响如图 3 所示。结果表明，经玻璃体内注射，这些融合蛋白对新生血管的生长具有显著的抑制作用。玻璃体内注射的作用效果要优于腹腔注射。同时，通过这组实验，

我们观察到 FP_7 的效果类似于 FP_3 两者都要好于 FP_1。在这个试验中，由于是玻璃体内注射，FP7 不需经体内血液循环。因此，其血液内稳定性较低的物质不会影响它的疗效。

实施例 5　融合蛋白对激光诱发的脉络膜新生血管生长的影响

根据发表的文献资料(American Journal Pathology 153,1641-1646,1998)，我们利用激光在大鼠的眼睛上建立能诱发眼底脉络膜新生血管生成的 AMD 模型。将 150 只左右的大鼠分成四组，对照组的 10 只大鼠通过皮下注射接受 Fc 蛋白（20 mg/kg），处理过的每 10 只大鼠分别通过皮下注射 20 mg/kg 的 FP_1、FP_3和 FP_7总共接受 5 次注射，分别在激光处理前一天和激光处理之后的第 3、6、9 和 12 天。在激光处理后第 15 天，大鼠通过静脉注射接受 50mg 荧光标记的右旋糖苷，然后经麻醉处理，将眼睛摘除，尽快剥离脉络膜，剖成扁平状或冷冻包埋做切片用于分析 CNV 病变情况。结果如图 4 表明，经融合蛋白处理小鼠的 CNV 面积都比对照（Fc）要小，其中 FP_3的效果要优于 FP_1和 FP_7。FP_7与 FP_1的抑制 CNV 生成方面效果相当。

实施例 6　融合蛋白在治疗眼疾中的应用

这些融合蛋白可以通过适当的方式，如玻璃体内注射或静脉给药，可用于治疗与病变新生血管生长相关的一系列眼科疾病，其中包括年龄相关性黄斑变性（AMD)、糖尿病视网膜病变(diabetic retinopathy)、糖尿病黄斑水肿(diabetic macular edema)和视网膜血管阻塞 (central retinal vein occlusion)。同时这些融合蛋白也可以和其他治疗方法一起结合使用，如和光敏药物（photocoagulation）或者与激光疗法结合使用，以降低在激光处理之后由新生血管生长引起的治疗失败机率。这些融合蛋白还可以和手术结合使用。如在视网膜移植之后，由于新生血管的生长而使视网膜移植的手术失败。如果在手术的同时，病人接受这些融合蛋白的治疗，就可以提高视网膜移植的成功率。AMD 病人在通过正常眼科检查之后，建立基本的基准线，然后通过玻璃体注射的方法将融合蛋白（如 FP_3或 FP_7）注入体内。经处理后病人将到医院接受观察和检查，以记录这些融合蛋白对 AMD 的影响。一般在接受治疗之后第 1、2、6、14、30 和 90 天分别检查一次。同时，病人可能需要接受多次治疗，可以是每两到八周注射一次。每次注射的量为每只眼睛 10 微克至 5 毫克范围内。

实施例 7 FP3 融合蛋白冻干制剂的制备

先配制好制剂缓冲液（formulation buffer），将合格的原液（drug substance）解冻后，用制剂缓冲液稀释到所需的蛋白浓度。进行过滤除菌后，用移液器/分装器按要求装量分装至洁净的西林瓶（规格：0.5ml/2ml）中，往瓶口加洁净的丁基橡胶塞（半压塞）。放入冷冻干燥机中，设定好合适的冻干曲线（包括预冻、冷冻、抽真空、升温各阶段的时间、温度、真空度等参数的设定），进行冻干。当冻干过程结束后，压紧胶塞，取出西林瓶，在胶塞上加铝塑盖，用轧盖器轧紧。往西林瓶上贴标签，装入纸盒，存放于适宜的温度。

实施例 8 FP3 融合蛋白溶液制剂的制备

先配制好制剂缓冲液（formulation buffer），将合格的原液（drug substance）解冻后，用制剂缓冲液稀释到所需的蛋白浓度。进行过滤除菌后，用移液器/分装器按要求装量分装至洁净的西林瓶（规格：5ml/20ml）中，往瓶口加洁净的丁基橡胶塞，塞紧。在丁基橡胶塞上加铝塑盖，用轧盖器轧紧。往西林瓶上贴标签，装入纸盒，存放于适宜的温度。

实施例 9 FP3 融合蛋白眼用制剂的制备

先配制好制剂缓冲液，将合格的原液（drug substance）解冻后，用制剂缓冲液稀释到所需的蛋白浓度。进行过滤除菌后，用移液器按要求装量（≤200μl）分装至洁净的西林瓶（规格：0.5ml）中，或用玻璃注射器（规格：1ml，带灰橡胶活塞、27 号针头）抽吸至要求装量（≤100μl）。对于西林瓶，在瓶口端加洁净的丁基橡胶塞，塞紧，在丁基橡胶塞上加铝塑盖，用轧盖器轧紧，往西林瓶上贴标签；对于注射器，在活塞处加装橡胶塞，在针头上加装灰橡胶罩，在橡胶罩外再加装硬塑套，用铝封袋（已印好标签）封装（另配有带螺纹的塑料活塞杆、白色法兰盘接头，用不同的铝封袋封装）。装入纸盒，存放于适宜的温度。

实施例 10 FP1 融合蛋白眼用制剂的制备，制备方法同实施例 9

实施例 11 FP2 融合蛋白眼用制剂的制备，制备方法同实施例 9

实施例 12 FP4 融合蛋白眼用制剂的制备，制备方法同实施例 9

实施例 13 FP5 融合蛋白眼用制剂的制备，制备方法同实施例 9

实施例 14 FP6 融合蛋白眼用制剂的制备，制备方法同实施例 9

实施例 15 FP7 融合蛋白眼用制剂的制备，制备方法同实施例 9

序列表

<110>成都康弘生物科技有限公司

<120> VEGF 受体融合蛋白在治疗眼睛疾病中的应用

<160>4

<210>1

<211>308

<212>PRT

<213>人工序列

<400>1

GlyArgProPheValGluMetTyrSerGluIleProGluIleIle 15

HisMetThrGluGlyArgGluLeuValIleProCysArgValThr 30

SerProAsnIleThrValThrLeuLysLysPheProLeuAspThr 45

LeuIleProAspGlyLysArgIleIleTrpAspSerArgLysGly 60

PheIleIleSerAsnAlaThrTyrLysGluIleGlyLeuLeuThr 75

CysGluAlaThrValAsnGlyHisLeuTyrLysThrAsnTyrLeu 90

ThrHisArgGlnThrAsnThrIleIleAspValValLeuSerPro 105

SerHisGlyIleGluLeuSerValGlyGluLysLeuValLeuAsn 120

CysThrAlaArgThrGluLeuAsnValGlyIleAspPheAsnTrp 135

GluTyrProSerSerLysHisGlnHisLysLysLeuValAsnArg 150

AspLeuLysThrGlnSerGlySerGluMetLysLysPheLeuSer 165

ThrLeuThrIleAspGlyValThrArgSerAspGlnGlyLeuTyr 180

ThrCysAlaAlaSerSerGlyLeuMetThrLysLysAsnSerThr 195

PheValArgValHisGluLysProPheValAlaPheGlySerGly 210

MetGluSerLeuValGluAlaThrValGlyGluArgValArgIle 225

ProAlaLysTyrLeuGlyTyrProProProGluIleLysTrpTyr 240

LysAsnGlyIleProLeuGluSerAsnHisThrIleLysAlaGly 255

HisValLeuThrIleMetGluValSerGluArgAspThrGlyAsn 270

TyrThrValIleLeuThrAsnProIleSerLysGluLysGlnSer 285

HisValValSerLeuValValTyrValProProAspLysThrHis 300

ThrCysProLeuCysProAlaPro 308

<210>2

<211>214

<212>PRT

<213>人工序列

<400>2

GlyArgProPheValGluMetTyrSerGluIleProGluIleIle 15

HisMetThrGluGlyArgGluLeuValIleProCysArgValThr 30

SerProAsnIleThrValThrLeuLysLysPheProLeuAspThr 45

LeuIleProAspGlyLysArgIleIleTrpAspSerArgLysGly 60

PheIleIleSerAsnAlaThrTyrLysGluIleGlyLeuLeuThr 75

CysGluAlaThrValAsnGlyHisLeuTyrLysThrAsnTyrLeu 90

ThrHisArgGlnThrAsnThrIleIleAspValValLeuSerPro 105

SerHisGlyIleGluLeuSerValGlyGluLysLeuValLeuAsn 120

CysThrAlaArgThrGluLeuAsnValGlyIleAspPheAsnTrp 135

GluTyrProSerSerLysHisGlnHisLysLysLeuValAsnArg 150

AspLeuLysThrGlnSerGlySerGluMetLysLysPheLeuSer 165

ThrLeuThrIleAspGlyValThrArgSerAspGlnGlyLeuTyr 180

ThrCysAlaAlaSerSerGlyLeuMetThrLysLysAsnSerThr 195

PheValArgValHisGluLysAspLysThrHisThrCysProLeu 210

CysProAlaPro 214

<210>3

<211>924

<212>DNA

<213>人工序列

<400>3

ggtagacctt tcgtagagat gtacagtgaa atccccgaaa ttatacacat gactgaagga 60

agggagctcg tcattccctg ccgggttacg tcacctaaca tcactgttac tttaaaaaag 120

tttccacttg acactttgat ccctgatgga aaacgcataa tctgggacag tagaaagggc 180

ttcatcatat caaatgcaac gtacaaagaa atagggcttc tgacctgtga agcaacagtc 240

aatgggcatt tgtataagac aaactatctc acacatcgac aaaccaatac aatcatagat 300

gtggttctga gtccgtctca tggaattgaa ctatctgttg gagaaaagct tgtcttaaat 360

tgtacagcaa gaactgaact aaatgtgggg attgacttca actgggaata cccttcttcg 420

aagcatcagc ataagaaact tgtaaaccga gacctaaaaa cccagtctgg gagtgagatg 480

aagaaatttt tgagcacctt aactatagat ggtgtaaccc ggagtgacca aggattgtac 540

acctgtgcag catccagtgg gctgatgacc aagaagaaca gcacatttgt cagggtccat 600

gaaaaacctt ttgttgcttt tggaagtggc atggaatctc tggtggaagc cacggtgggg 660

gagcgtgtca gaatccctgc gaagtacctt ggttacccac ccccagaaat aaaatggtat 720

aaaaatggaa taccccttga gtccaatcac acaattaaag cggggcatgt actgacgatt 780

atggaagtga gtgaaagaga cacaggaaat tacactgtca tccttaccaa tcccatttca 840

aaggagaagc agagccatgt ggtctctctg gttgtgtatg tcccaccgga caaaactcac 900

acatgcccac tgtgcccagc acct 924

<210>4

<211>642

<212>DNA

<213>人工序列

<400>4

ggtagacctt tcgtagagat gtacagtgaa atccccgaaa ttatacacat gactgaagga 60

agggagctcg tcattccctg ccgggttacg tcacctaaca tcactgttac tttaaaaaag 120

tttccacttg acactttgat ccctgatgga aaacgcataa tctgggacag tagaaagggc 180

ttcatcatat caaatgcaac gtacaaagaa atagggcttc tgacctgtga agcaacagtc 240

aatgggcatt tgtataagac aaactatctc acacatcgac aaaccaatac aatcatagat 300

gtggttctga gtccgtctca tggaattgaa ctatctgttg gagaaaagct tgtcttaaat 360

tgtacagcaa gaactgaact aaatgtgggg attgacttca actgggaata cccttcttcg 420

aagcatcagc ataagaaact tgtaaaccga gacctaaaaa cccagtctgg gagtgagatg 480

aagaaatttt tgagcacctt aactatagat ggtgtaaccc ggagtgacca aggattgtac 540

acctgtgcag catccagtgg gctgatgacc aagaagaaca gcacatttgt cagggtccat 600

gaaaaagaca aaactcacaca tgcccactgt gcccagcac ct 642

<210>5

<211>552

<212>PRT

<213>人工序列

<400>5

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu
1 5 10 15 20

Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile
25 30 35 40

Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile
45 50 55 60

Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
65 70 75 80

Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu
85 90 95 100

Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln
105 110 115 120

Thr Asn Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly
125 130 135 140

Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn
145 150 155 160

Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg Asp Leu Lys Thr
165 170 175 180

Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg
185 190 195 200

Ser Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser
205 210 215 220

Thr Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu
225 230 235 240

Val Glu Ala Thr Val Gly Glu Arg Val Arg leu Pro Ala Lys Tyr Leu Gly Tyr Pro Pro
245 250 255 260

Pro Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala
265 270 275 280

Gly His Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile
285 290 295 300

Leu Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val Val Tyr Val
305 310 315 320

Pro Pro Gly Pro Gly Asp Lys Thr His Thr Cys Pro Leu Cys Pro Ala Pro Glu Leu Leu
325 330 335 340

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
345 350 355 360

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
365 370 375 380

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
385 390 395 400

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
405 410 415 420

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
425 430 435 440

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
445 450 455 460

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
465 470 475 480

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Ala Thr Pro
485 490 495 500

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
505 510 515 520

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
525 530 535 540

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
545 550 552

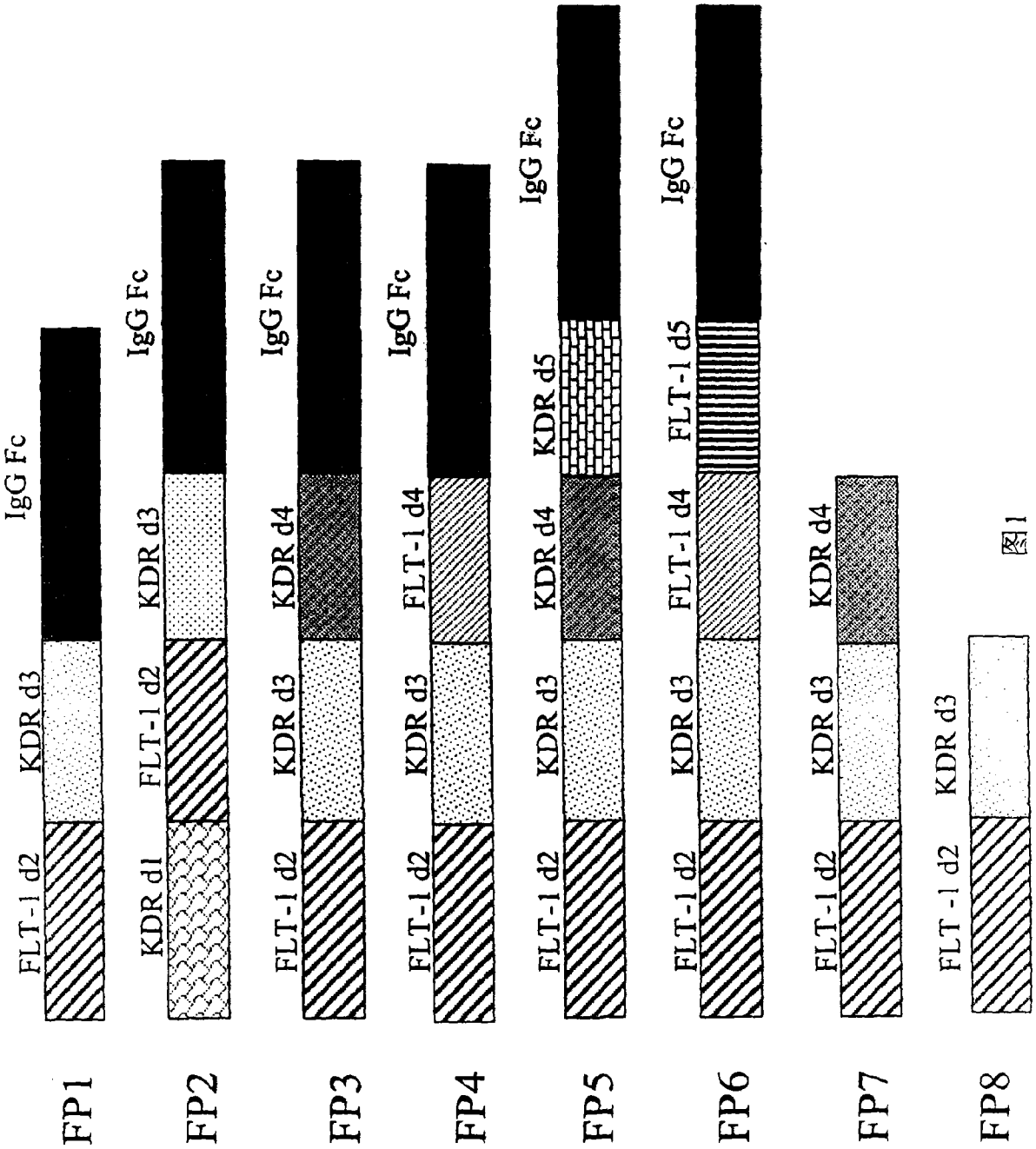


图1

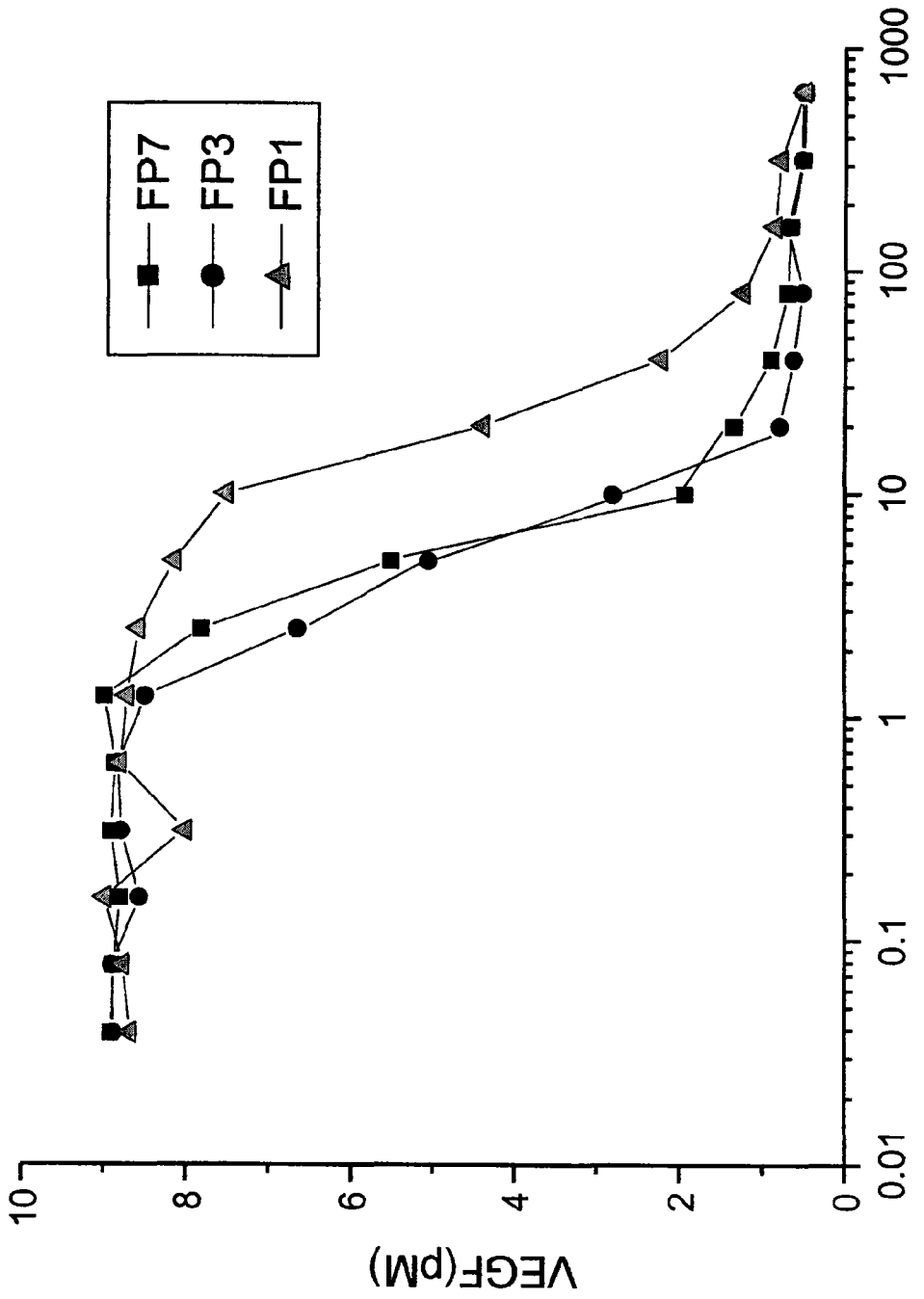


图2

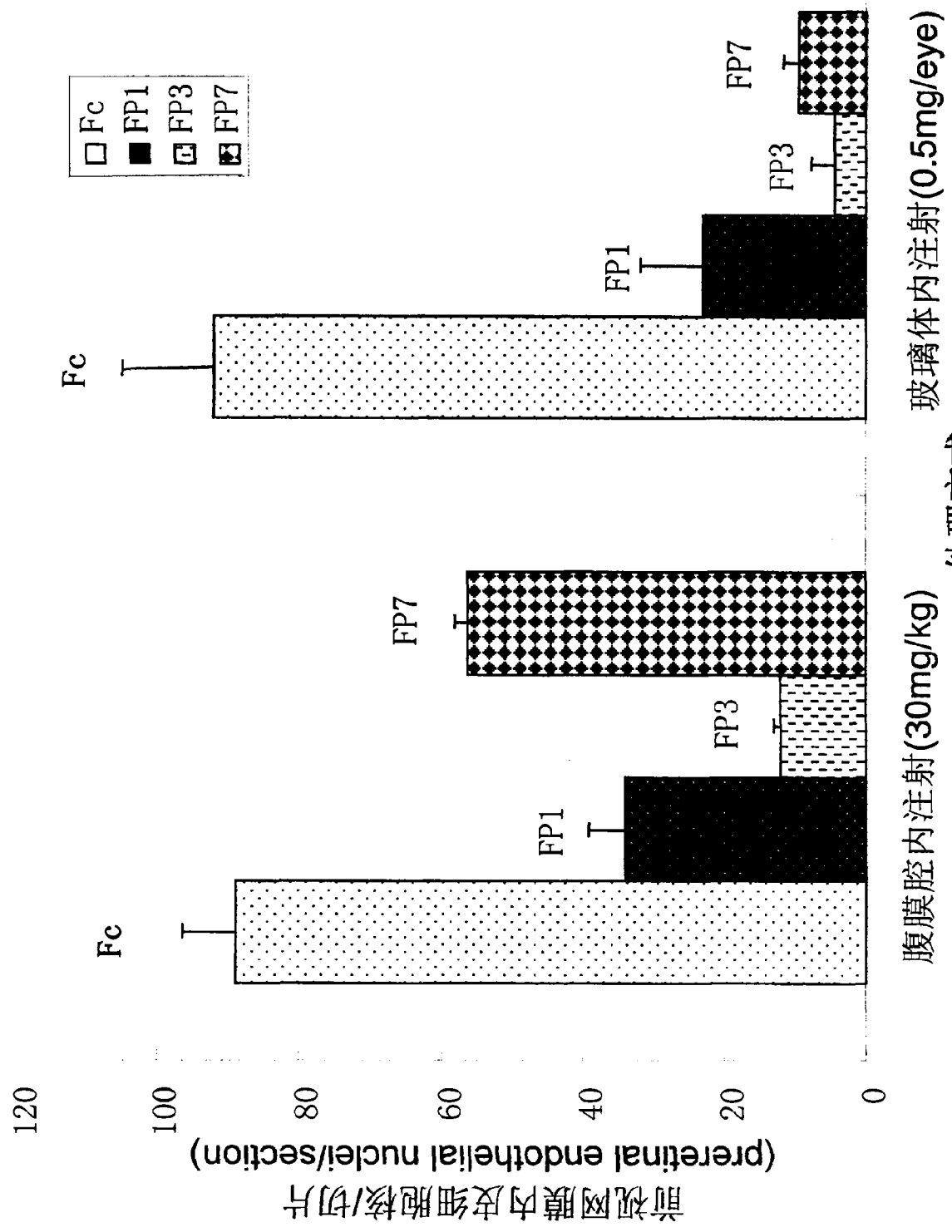


图3

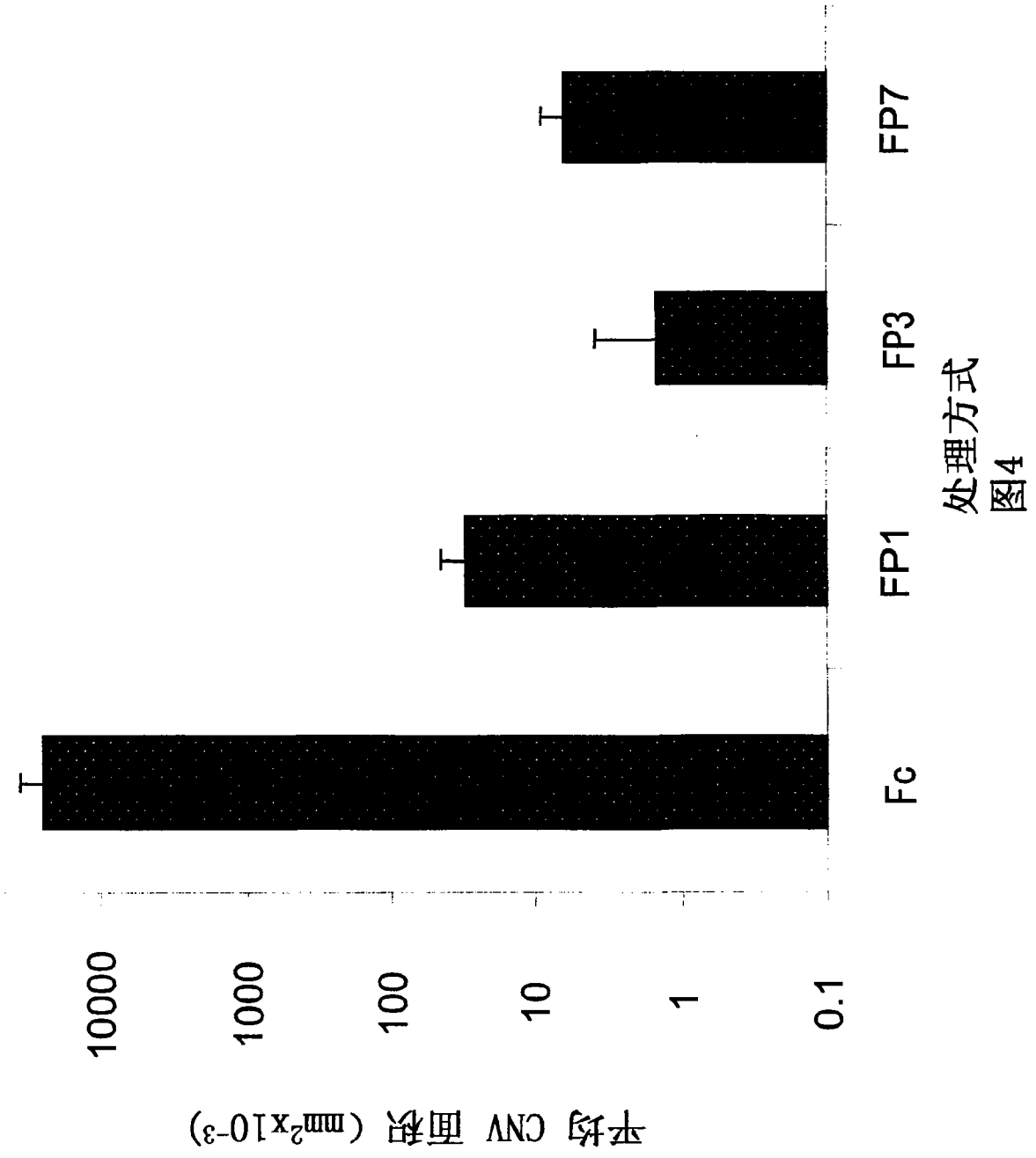


图4

Notice

ABSTRACT CN100567325C

[0001]

13 The present invention relates to two VEGF receptor fusion proteins, its preparation method and its application in the treatment of eye diseases, including age-related macular degeneration, diabetic retinopathy, diabetic xanthoma glands, and growth of new blood vessels. Treatment failures caused by laser coagulation, surgical retina transplantation.

CN100567325C VEGF receptor fusion protein and its use in preparation of medicament for treating eye disease

This application is a divisional application for a Chinese invention patent application with an application date of March 31, 2006, application number 200610066257.2, and an invention titled "Application of VEGF Receptor Fusion Protein in Treatment of Eye Diseases."

Technical field:

The invention relates to the application of VEGF receptor fusion protein in the treatment of various ophthalmic diseases caused by the growth of new blood vessels.

Background technique:

The retinal vasculature and chorodial vasculature are important components of the retina. Abnormal changes in the structure or function of blood vessel walls caused by trauma or disease are the main causes of vision loss or loss. For example, diabetic retinopathy (diabetic retinopathy) is due to diabetes and leads to retinal blood vessel proliferation, which in turn causes retinal detachment. It is the main cause of vision loss. It may also cause the growth of new blood vessels in the retina after eye injury or recovery after surgery. This type of retinal vascular hyperplasia is also an important cause of vision loss or blindness (Nature 438: 932-938, 2005).

Age-related macular degeneration (AMD) is a disease caused by the degradation of cells or tissues in the central area of the retina and the proliferation of blood vessels. Can be divided into dry and wet. Among them, wet AMD is the most common form of choroidal neovascularization, and it is also the main cause of blindness in the eyes.

Vascular endothelial growth factor (VEGF) is a protein that specifically regulates the growth of new blood vessels (Am. J. Pathol. 167: 1451-1459, 2005). VEGF stimulates endothelial cells to divide and proliferate, which in turn promotes the growth of new blood vessels to provide nutrients and oxygen to tissue cells. Many studies have shown that once the photoreceptor cells of the eye's retina begin to shrink due to nutritional deficiencies (called "ischemic atrophy"), the concentration of VEGF in the retina begins to increase, thereby promoting the growth of new blood vessels. This process is called "angiogenesis" (angiogenesis). In the eye, the new blood vessels are different from normal blood vessels in shape, the lumen is irregular, and the wall is mostly leaky. This abnormal proliferation of highly permeable or leaking blood vessels often leads to scarring on the retina, and further shedding can occur, affecting vision.

Many studies have shown high levels of VEGF expression in the choroid tissue of patients with wet AMD (Invest. Opthal. Vis. Sci 37: 855-868, 1996; Microvascular Res. 64: 162-169, 2002). Due to the correlation between VEGF expression levels and wet AMD, VEGF can be used as a biochemical indicator for the diagnosis of AMD (Br. J. Opthalmol. 88: 809-815, 2004).

Some VEGF inhibitors can block the interaction between VEGF and VEGF receptors (flt-1, KDR, etc.) on endothelial cells, thereby preventing VEGF-mediated information transmission and inhibiting neovascularization caused by high expression of VEGF Grow to achieve the purpose of preventing and stopping bleeding on the retina. Such VEGF inhibitors include Macugen (pegaptanib sodium), Lucentis, VEGF-Trap, Avastin (bevacizumab) and AdPEDF. Macugen and Avastin have been approved by the US National Food and Drug Administration (FDA) for listing.

Summary of the invention:

The present invention provides a class of fusion proteins that inhibit VEGF and can be used to treat various ophthalmic diseases caused by the growth of new blood vessels. The diseases include (but are not limited to) the eye diseases including age-related macular degeneration, diabetic retinopathy, diabetic macular tumor glands,

and treatment failures caused by neovascularization such as laser coagulation, surgical retina transplantation. In the present invention, the fusion protein refers to a macromolecular compound, especially a recombinant fusion protein of VEGF receptor. More specifically, the VEGF inhibitors of the present invention are FP1, FP2, FP3, FP4, FP5, FP6 and the present invention described in the Chinese patent application "Fusion Proteins Inhibiting Angiogenesis and Their Uses" (application number CN200510073595.4) Any one of FP7 and FP8 provided. The amino acid sequences of the fusion proteins of FP1, FP2, FP3, FP4, FP5, and FP6 are in the above patent specification, and the amino acid sequences of FP7 and FP8 are in sequence 1 and sequence 2 in the sequence listing of the present invention.

The VEGF receptor fusion protein of the present invention can be prepared by the method in the above patent specification, where FP1, FP2, FP3, FP4, FP5, FP6 are known substances, FP7 and FP8 are new substances, and for new substances, the present invention An example of its preferred preparation method is provided, and its preparation principle is the same as the above patent specification.

The products made by the recombinant technology of the present invention are purified to achieve pharmaceutical purity, and then prepared into pharmacentical preparations according to the needs of the preparations. These preparations should be particularly suitable for intravenous administration, intravitreal injection, intraperitoneal injection, and subcutaneous injection For injection and local intraocular administration, the preparation process can be completed by the conventional preparation method of pharmaceutical preparations, preferably a solution preparation or a dry powder preparation. As a dry powder preparation, the dry powder is dissolved into a solution when used. The fusion protein preparation of the present invention can be added with a pharmaceutically acceptable carrier if necessary, and the carrier can be any pharmaceutical carrier suitable for the preparation form of the present invention, preferably selected from: sodium phosphate and sodium succinate succinate, histidine, mannitol, trehalose (trehalose dihydrate), polysorbate 20 (polysorbate 20), sodium chloride (sodium chloride), sucrose (sucrose), trishydroxymethylaminomethane (trometamol) or lactose, the above formulation buffer (formulation buffer) should contain a pH buffer system such as phosphate, citrate, acetate, succinate, trihydroxy Methylaminomethane (trometamol, also known as Tris) or histidine (histidine), etc., the pH range is 3 to 9; may contain osmotic pressure regulators such as sodium chloride (sodium chloride), glucose (dextrose) Etc .; may contain stabilizers such as amino acids, glycerol, cyclodextrin, sucrose, trehalose dihydrate, etc .; may contain preservatives such as thimerosal Sodium bisulfite (sodium bisulfite), phenylethanol (benzylalcohol) and the like. For lyophilized formulations, it may contain excipients such as mannitol, etc .; for solution formulations, it may contain surfactants such as polysorbate (polysorbate 20 or 80), sodium dodecyl sulfonate (SDS), etc. . The concentration of fusion protein ranges from 0.01 mg / ml to 1000 mg / ml. Its dosage depends on clinical needs. In addition, preservatives, stabilizers, co-solvents and other auxiliary components can be added to the formulation as needed, and can be selected from any suitable auxiliary components of pharmacy. The solvent is selected from water or other isotonic solutions, buffer solutions, etc.

In the present invention, the administration method of VEGF inhibitor is also introduced. These inhibitors can be administered to patients through a variety of different routes of administration, including (but not limited to) intravenous administration, intravitreal injection, and can also be administered to patients by eye drops in a certain dosage form to achieve the purpose of treating eye diseases .

The present invention unexpectedly found that the VEGF inhibitor in the form of a fusion protein of the present invention has superior ophthalmic treatment effect than the prior art, and has good stability, high safety, low side effects, and excellent effects. The present invention proves the present invention through experimental data See the examples for specific data.

BRIEF DESCRIPTION:

Figure 1: Schematic diagram of the eight fusion proteins of the present invention

Figure 2: Comparison of the affinity of the fusion protein to VEGF

Figure 3: Effect of fusion protein on neovascularization caused by retinal ischemic atrophy

Figure 4: Effect of fusion protein on choroidal neovascularization

detailed description:

The following examples further illustrate the present invention, but it is not intended to limit the present invention.

Example 1, construction of FP7

The fusion protein FP7 was extracted from HUVEC cells by primers flt-1D2 (F), flt-1D2 (R), KDR D3 (F) and KDRD3-4 (R) (see patent application, application number CN200510073595.4) The amplified flt-1 and KDR gene fragments synthesized on the cDNA synthesized by using mRNA as a template are recombined. The specific conditions are: denaturation 95 °, 30 minutes, annealing 56 °, 45 seconds, extension 72 °, 2 minutes, PCR amplification, 30 cycles to obtain flt-1 and KDR IgG-like domain PCR products. Using TA cloning kit, clone the PCR product into the PCR2.1 plasmid, and transfect E. coli, select white colonies, add LB medium, and culture overnight. The plasmid was extracted with Qiangen plasmid extraction kit, digested and sequenced. Using splicing PCR (Sewing PCR) method, the flt-1 fragment, KDR fragment and ribonucleotides of the partial sequence of the IgG hinge region are connected together. Design EcoR1 restriction sites in the primers at both ends. After digestion with EcoR1, the final PCR product was purified by Qiangen purification kit and inserted into pcDNA3.1 plasmid. The recombinant plasmid was transfected into E. coli, positive colonies were selected, added to LB medium, and cultured overnight. Qiangen plasmid was digested after plasmid extraction, and sequenced for identification. If the confirmed plasmid is then transfected into CHO cells, a cell line stably expressing the fusion protein FP7 must be obtained. The specific nucleotide sequence of FP7 is shown in Sequence Listing 3. At the C-terminus of the fusion protein, a partial sequence of the hinge region is retained.

Example 2, construction of FP8

The fusion protein FP8 is directly amplified by PCR using FP7 as a template. The primers used in PCR are flt-1D2 (F) and KDR D3-hing (R). The sequence of the latter is: 5'-aggtgctgggcacagtgggcatgtgtgagttttgtctttttcatggaccctgacaaatg. It includes a sequence complementary to the third immunoglobulin-like region of KDR and a partial nucleotide sequence of the human IgG Fc hinge region. The method of PCR amplification and gene cloning is the same as in Example 1. Finally, the PcDNA3.1 plasmid inserted with FP8 was transfected into CHO cells, and a stable cell line was obtained for protein expression. The amino acid sequence of FP8 is shown in Sequence Listing 2, and the nucleotide sequence is shown in Sequence Listing 4.

Example 3 Experiment of binding affinity of fusion protein to VEGF

The present invention determines the ability of various fusion proteins to bind VEGF by measuring the amount of VEGF. In this experiment, a certain amount of VEGF (10PM) was added to the test tube, and then the diluted various fusion proteins containing different amounts were added to the test tube containing VEGF, after mixing, in a 37 ° incubator Save for an hour. One hour later, the free VEGF in the test tube was measured by the VEGF detection kit (VEGF assay kit) provided by R & D Systems. The measured results are processed by the software to obtain the results shown in Figure 2. Figure 2 shows that FP1, FP3 and FP7 can effectively bind to VEGF, and their binding affinity can be expressed by IC50, which are 11.2PM and 4.3 respectively. PM and 4.1PM. This experiment proves that FP3 and FP7 have similar binding ability to VEGF in vitro, and both are higher than FP1.

The results of this experiment further illustrate that the amino acid sequence of the fourth immunoglobulin-like region of KDR can enhance the fusion protein's ability to bind VEGF.

Example 4 Experimental results of fusion protein preventing the formation of neovascularization caused by retinal ischemic atrophy

The seven-day-old pups were placed in an incubator containing high oxygen partial pressure (75% ± 2%), and the temperature was controlled at 23 ° C ± 2 ° C under daylight illumination. After a few days of culture under this condition, no angiogenesis will occur in the center of the retina, and after five days, the pups are returned to the incubator with normal oxygen partial pressure. Due to the relatively low oxygen partial pressure concentration in the chamber, hypoxic conditions are generated in the retina of young rats, thereby stimulating the generation of neovascular reactions similar to diabetic retinopathy and other ischemic atrophic retinopathy.

Using this model, the role of three fusion proteins (FP1, FP3, and FP7) in neovascularization associated with ischemic atrophic retinopathy can be evaluated.

Place the pups in a high oxygen partial pressure incubator and bring them back to the normal oxygen partial pressure incubator after five days. The pups were divided into five groups of 10 mice, and the fusion protein was injected intraperitoneally at a dose of 30 mg / kg one day later, once every two days, for a total of 4 injections. The control mice were injected with the same amount of Fc protein. After the end of the treatment period, 6 mice were taken from each group and fluorescein FAM was injected into the heart. After 10 minutes, the retinas of the young mice were removed for analysis of neovascularization. During the operation, the retina was laid flat, and the neovascularization and fluorescence leakage were observed under a fluorescent microscope. The eyes of the remaining four mice in each group were embedded in paraffin and stained with H & E after sectioning. The number of vascular endothelial cell nuclei was counted under a microscope to determine the effect of fusion protein on neovascularization (Investigative Ophthalomogy visual science 43, 1994-2000, 2002). The results are shown in Figure 3. The retinas of young mice receiving Fc protein injection all showed severe lesions. A large number of disordered blood vessels can be observed on the inner retinal membrane. There is no obvious blood vessel growth on the retina of the treated mice treated with fusion protein (Figure 3). Among them, FP3 is the most effective and FP7 is also more effective, but its effect is similar to FP1, the main reason may be due to the deletion of Fc fragments And affect its stability in the body. FP1 and FP7 have similar effects.

At the same time, we also tested the effect of these fusion proteins on neovascularization when administered intravitreally. The same animal model was used for the test. One day after the animal returned to the incubator with normal oxygen partial pressure, intravitreal injection was performed at 0.5 mg per eye, and each animal received only one treatment. On the seventh day after administration, the animal's retina was collected and processed as described above. The effect of fusion protein on neovascularization is shown in Figure 3. The results showed that after intravitreal injection, these fusion proteins had a significant inhibitory effect on the growth of neovascularization. The effect of intravitreal injection is better than intraperitoneal injection. At the same time, through this set of experiments, we observed that the effect of FP7 is similar to FP3 and both are better than FP1. In this test, due to intravitreal injection, FP7 does not need to circulate through the body. Therefore, substances with lower stability in the blood will not affect its efficacy.

Example 5 Effect of fusion protein on laser-induced choroidal neovascularization

According to published literature (American Journal Pathology 153, 1641-1646, 1998), we used lasers to build an AMD model that can induce fundus choroidal neovascularization in the eyes of rats. About 150 rats were divided into four groups. The 10 rats in the control group received Fc protein (20 mg / kg) by subcutaneous injection. Each 10 rats treated were subcutaneously injected with 20 mg / kg of FP1, FP3 and FP3. FP7 received a total of 5 injections, one day before laser treatment and 3, 6, 9 and 12 days after laser treatment. On the 15th day after laser treatment, rats received 50 mg of fluorescently labeled dextran by intravenous injection, and then under anesthesia treatment, the eyes were removed, the choroid was peeled off as soon as possible, cut into flat or frozen embedded sections for analysis of CNV lesions Happening. The results shown in Figure 4 show that the CNV area of mice treated with fusion protein is smaller than that of the control (Fc), and the effect of FP3 is better than that of FP1 and FP7. FP7 is equivalent to FP1 in inhibiting CNV production.

Example 6 Application of fusion protein in the treatment of eye diseases

These fusion proteins can be used by appropriate methods, such as intravitreal injection or intravenous administration, to treat a range of ocular diseases related to the growth of diseased neovascularization, including age-related macular degeneration (AMD), diabetic retinopathy (diabetic retinopathy)), Diabetic macular edema (diabeticmacular edema) and retinal vascular occlusion (centralretinal veinocclusion). At the same time, these fusion proteins can also be used in combination with other treatment methods, such as photocoagulation or laser therapy, to reduce the chance of treatment failure caused by neovascularization after laser treatment. These fusion proteins can also be used in conjunction with surgery. For example, after retinal transplantation, the operation of retinal transplantation fails due to the growth of new blood vessels. If the patient receives these fusion proteins at the same time as the operation, the success rate of retinal transplantation can be improved. AMD patients establish a basic baseline after passing a normal eye examination, and then inject the fusion protein (such as FP3 or FP7) into the body by vitreous injection. After the treatment, the patient will go to the hospital for observation and examination to record the impact of these fusion proteins on AMD. It is usually checked on days 1, 2, 6, 14, 30 and 90 after receiving treatment. At the same time, patients may need to receive multiple treatments, which can be given every two to eight weeks. The amount of each injection is in the range of 10 μg to 5 mg per eye.

Example 7 Preparation of FP3 fusion protein lyophilized preparation

First prepare the formulation buffer (formulation buffer), thaw the qualified stock solution (drug substance), and then use the formulation buffer to dilute to the desired protein concentration. After filtration and sterilization, use a pipette / dispenser to dispense into a clean vial (specification: 0.5ml / 2ml) according to the required amount, and add a clean butyl rubber stopper (half pressure stopper) to the bottle mouth . Put it in the freeze dryer, set the appropriate freeze-drying curve (including the setting of parameters such as time, temperature, vacuum degree of pre-freezing, freezing, evacuation, temperature rise), and freeze-dry. When the freeze-drying process is over, compress the rubber stopper, take out the vial, add an aluminum-plastic cap to the rubber stopper, and tighten it with a capping device. Put a label on the vial, put it in a carton, and store at a suitable temperature.

Example 8 Preparation of FP3 fusion protein solution preparation

First prepare the formulation buffer (formulation buffer), thaw the qualified stock solution (drug substance), and then use the formulation buffer to dilute to the desired protein concentration. After filtration and sterilization, use a pipette / dispenser to dispense into a clean vial (specification: 5ml / 20ml) according to the required amount, and add a clean butyl rubber stopper to the bottle to tightly stop. Add an aluminum-plastic cap to the butyl rubber plug and tighten it with a capping device. Put a label on the vial, put it in a carton, and store at a suitable temperature.

Example 9 Preparation of FP3 fusion protein ophthalmic preparation

Prepare the preparation buffer first, thaw the qualified substance (drug, substance), and then use the preparation buffer to dilute to the desired protein concentration. After filtration and sterilization, use a pipette to fill the cleaned vial (≤200μl) into a clean vial (specification: 0.5ml), or use a glass syringe (specification: 1ml, with gray rubber piston, 27 gauge needle)) Pump to the required volume (≤100μl). For the vial, add a clean butyl rubber stopper to the end of the bottle, plug tightly, add an aluminum-plastic cap to the butyl rubber stopper, use a capping machine to tighten, and label the vial; for syringes, add a stopper to the piston Install a rubber plug, add a gray rubber cover to the needle, and add a hard plastic sleeve outside the rubber cover, and encapsulate it with an aluminum sealing bag (with a printed label) (also equipped with a threaded plastic piston rod, white flange) The joints are sealed in different aluminum bags). Put in a carton and store at a suitable temperature.

Example 10 Preparation of FP1 fusion protein ophthalmic preparation, the preparation method is the same as Example 9

Example 11 Preparation of FP2 fusion protein ophthalmic preparation, the preparation method is the same as Example 9

Example 12 Preparation of FP4 fusion protein ophthalmic preparation, the preparation method is the same as Example 9

Example 13 Preparation of FP5 fusion protein ophthalmic preparation, the preparation method is the same as Example 9

Example 14 Preparation of FP6 fusion protein ophthalmic preparation, the preparation method is the same as Example 9

Example 15 Preparation of FP7 fusion protein ophthalmic preparation, the preparation method is the same as Example 9

Sequence Listing

(110) Chengdu Kanghong Biological Technology Co., Ltd.

(120) Application of VEGF receptor fusion protein in preparation of medicine for treating eye diseases

(160)4

(210)1

(211)308

(212) PRT

(213) Artificial sequence

(400)1

GlyArgProPheValGluMetTyrSerGluIleProGluIleIle 15

HisMetThrGluGlyArgGluLeuValIleProCysArgValThr30

SerProAsnIleThrValThrLeuLysLysPheProLeuAspThr45

LeuIleProAspGlyLysArgIleIleTrpAspSerArgLysGly60

PheIleIleSerAsnAlaThrTyrLysGluIleGlyLeuLeuThr75

CysGluAlaThrValAsnGlyHisLeuTyrLysThrAsnTyrLeu90

ThrHisArgGluThrAsnThrIleIleAspValValLeuSerPro105

SerHisGlyIleGluLeuSerValGlyGluLysLeuValLeuAsn120

CysThrAlaArgThrGluLeuAsnValGlyIleAspPheAsnTrp135

GluTyrProSerSerLysHisGlnHisLysLysLeuValAsnArg150

AspLeuLysThrGlnSerGlySerGluMetLysLysPheLeuSer165

ThrLeuThrIleAspGlyValThrArgSerAspGlnGlyLeuTyr180

ThrCysAlaAlaSerSerGlyLeuMetThrLysLysAsnSerThr195

PheValArgValHisGluLysProPheValAlaPheGlySerGly210

MetGluSerLeuValGluAlaThrValGlyGluArgValArgIle225

ProAlaLysTyrLeuGlyTyrProProProGluIleLysTrpTyr240

LysAsnGlyIleProLeuGluSerAsnHisThrIleLysAlaGly255

HisValLeuThrIleMetGluValSerGluArgAspThrGlyAsn270

TyrThrValIleLeuThrAsnProIleSerLysGluLysGlnSer285

HisValValSerLeuValValTyrValProProAspLysThrHis300

ThrCysProLeuCysProAlaPro308

(210)2

(211)214

(212) PRT

(213) Artificial sequence

(400)2

GlyArgProPheValGlnMetTyrSerGluIleProGluIleIle 15

HisMetThrGluGlyArgGluLeuValIleProCysArgValThr30

SerProAsnIleThrValThrLeuLysLysPheProLeuAspThr45

LeuIleProAspGlyLysArgIleIleTrpAspSerArgLysGly60

PheIleIleSerAsnAlaThrTyrLysGluIleGlyLeuLeuThr75

CysGluAlaThrValAsnGlyHisLeuTyrLysThrAsnTyrLeu90

ThrHisArgGlnThrAsnThrIleIleAspValValLeuSerPro105

SerHisGlyIleGluLeuSerValGlyGluLysLeuValLeuAsn120

CysThrAlaArgThrGluLeuAsnValGlyIleAspPheAsnTrp135

GluTyrProSerSerLysHisGlnHisLysLysLeuValAsnArg150

AspLeuLysThrGlnSerGlySerGluMetLysLysPheLeuSer165

ThrLeuThrIleAspGlyValThrArgSerAspGlnGlyLeuTyr180

ThrCysAlaAlaSerSerGlyLeuMetThrLysLysAsnSerThr195

PheValArgValHisGluLysAspLysThrHisThrCysProLeu210

CysProAlaPro214

(210)3

(211)924

(212) DNA

(213) Artificial sequence

(400)3

ggtagacctt tcgtagagat gtacagtgaa atccccgaaa ttatacacat gactgaagga 60

agggagctcg tcattccctg ccgggttacg tcacctaaca tcactgttac tttaaaaaag 120

tttccacttgacactttgatccctgatggaaaacgcataatctgggacagtagaaagggc180

ttcatcatatcaaatgcaacgtacaaagaaataggggcttctgacctgtgaagcaacagtc240

aatgggcatttgtataagacaaactatctcacacatcgacaaaccaatacaatcatagat300

gtggttctga gtccgtctca tggaattgaa ctatctgttg gagaaaagct tgtcttaaat 360

tgtacagcaa gaactgaactaaatgtggggattgacttcaactgggaataccttcttcg420

aagcatcagcataagaaacttgtaaaccgagacctaaaaacccagtctgggagtgagatg480

aagaaatttt tgagcacctt aactatagat ggtgtaaccc ggagtgacca aggattgtac 540

acctgtgcag catccagtgg gctgatgacc aaagaagaaca gcacatttgt cagggtccat 600

gaaaaacctttgttgcttttggaagtggcatggaatctctggtggaagccacggtgggg660

gagcgtgtca gaatccctgc gaagtacctt ggttacccac ccccagaaat aaaatggtat 720

aaaaatggaa tacccccttgagtccaatcacacaattaaagcggggcatgtactgacgatt780

atggaagtga gtgaaagaga cacaggaaat tacactgtca tccttaccaa tcccatttca 840

aaggagaagcagagccatgtggtctctctggttgtgtatgtccccaccggacaaaactcac900

acatgc ccac tgtgcccagc acct 924

(210)4

(211)642

(212) DNA

(213) Artificial sequence

(400)4

ggtagacctt tcgtagagat gtacagtgaa atccccgaaa ttatacacat gactgaagga 60

agggagctcg tcattccctg ccgggttacg tcacctaaca tcactgttac tttaaaaaag 120

tttccacttgacactttgatccctgatggaaaacgcataatctgggacagtagaaagggc180

ttcatcatatcaaatgcaacgtacaaagaaatagggcttctgacctgtgaagcaacagtc240

aatgggcatttgtataagacaaactatctcacacatcgacaaaccaatacaatcatagat300

gtggttctga gtccgtctca tggaattgaa ctatctgttg gagaaaagct tgtcttaaat 360

tgtacagcaa gaactgaactaaatgtggggattgacttcaactgggaatacccttcttcg420

aagcatcagcataagaaacttgtaaaccgagacctaaaaacccagtctgggagtgagatg480

aagaaatttttgagcaccttaactatagatggtgtaacccggggtgacca aggattgtac540

acctgtgcag catccagtgg gctgatgacc aaagaagaaca gcacatttgt cagggtccat 600

gaaaaagacaaaactcacacatgcccactgtgcccagcacct642

CN100567325C VEGF receptor fusion protein and its use in preparation of medicament for treating eye disease

1.Receptor fusion protein FP7 of vascular endothelial growth factor VEGF, its amino acid sequence is shown as sequence 1 in the sequence table.

2.The nucleotide sequence of the gene encoding the vascular endothelial growth factor VEGF receptor fusion protein FP7 is shown as sequence 3 in the sequence table.

3.The expression vector containing the gene of vascular endothelial growth factor VEGF receptor fusion protein FP7 according to claim 2.

4.A method for preparing the VEGF receptor fusion protein FP7 according to claim 1, comprising the steps of constructing a vector containing a fusion protein gene using genetic recombination technology, introducing the vector into a genetically engineered cell, and expressing the fusion protein.

5.A pharmaceutical composition comprising a therapeutically effective amount of the VEGF receptor fusion protein FP7 of claim 1 and optionally one or more pharmaceutically acceptable carriers.

6.The pharmaceutical composition according to claim 5, wherein the carrier is selected from one or more of buffers, stabilizers, preservatives, co-solvents, excipients, and osmotic pressure adjusting agents between PH3-9.

7.The pharmaceutical composition according to claim 6, wherein the carrier is selected from phosphate buffer, succinate buffer, histidine buffer, mannitol, trehalose, polysorbate, sodium chloride, sucrose, One or more of hydroxymethylaminomethane or lactose.

8.The pharmaceutical composition according to any one of claims 5-7, which is prepared into a pharmaceutical preparation suitable for use, the preparation is selected from a lyophilized preparation or a solution preparation.

9.The pharmaceutical composition according to claim 8, wherein the administration method of the formulation is selected from intravitreal injection, intravenous injection, intraperitoneal injection, subcutaneous injection, or eye drop administration.

10.Use of the fusion protein FP7 according to claim 1, the gene according to claim 2 or the pharmaceutical composition according to any one of claims 5-9 in the preparation of a medicament for the treatment of ocular diseases related to neovascularization, wherein The ocular diseases related to neovascularization are selected from age-related macular degeneration, diabetic retinopathy, diabetic macular edema, retinal vein occlusion, and treatment failure caused by neovascularization.

11.The use according to claim 10, wherein the treatment failure caused by neovascularization is selected from laser coagulation and surgical retina transplantation.

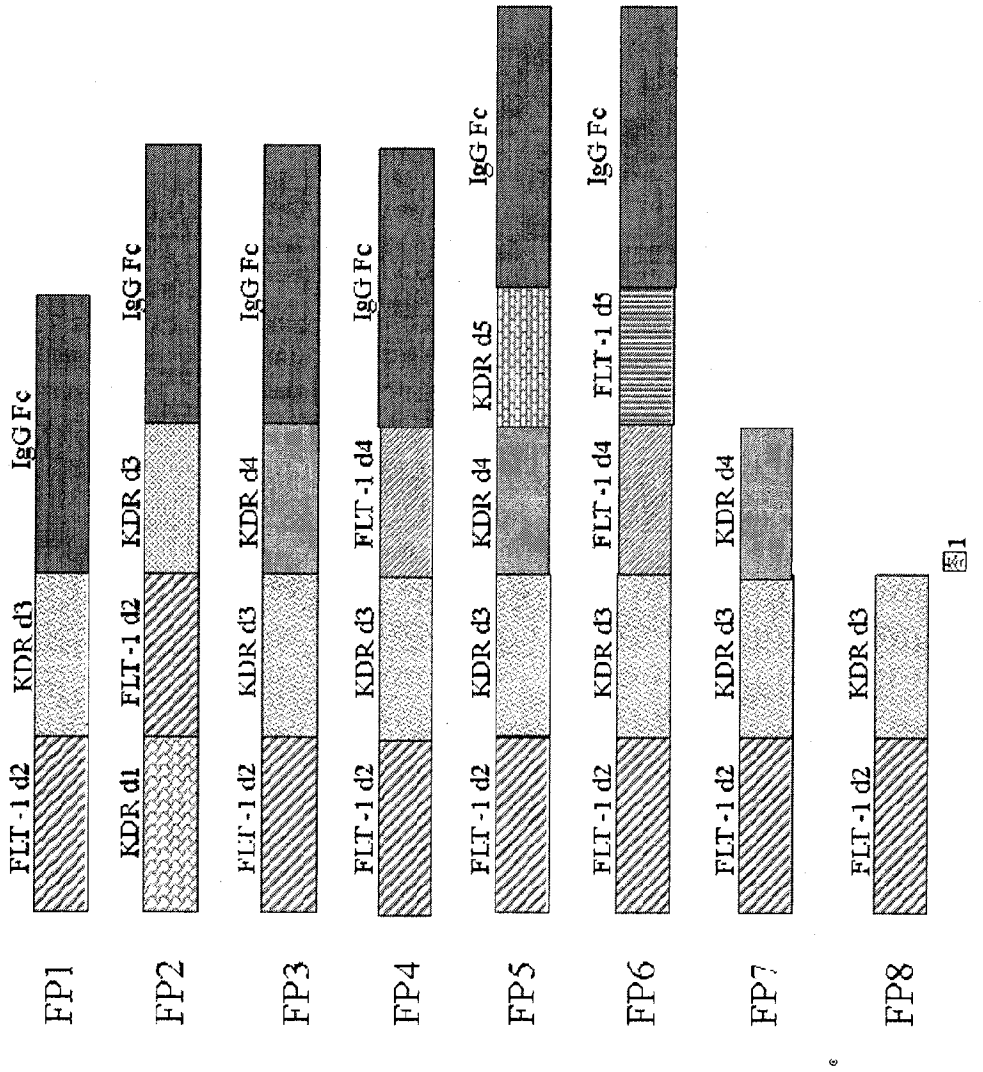


图1

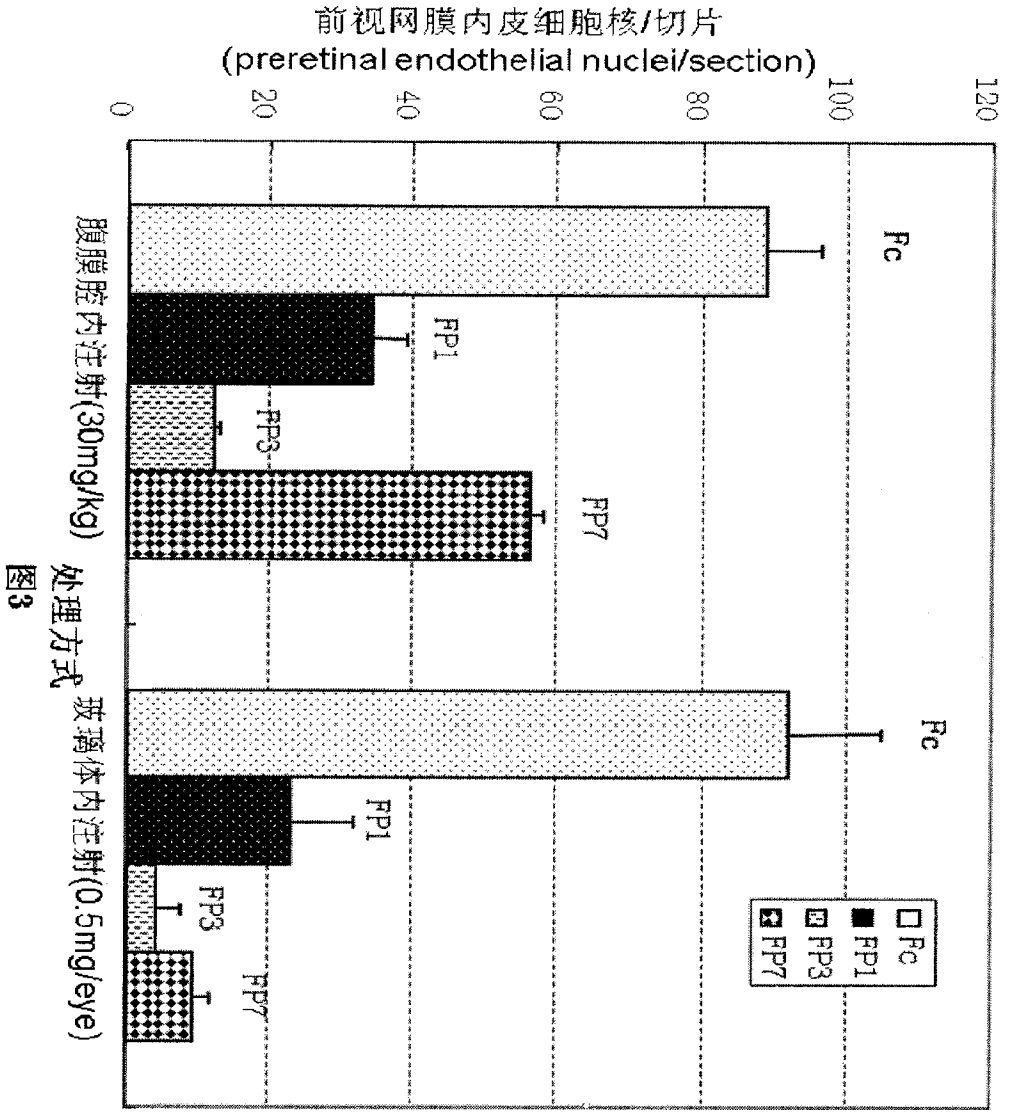


图3

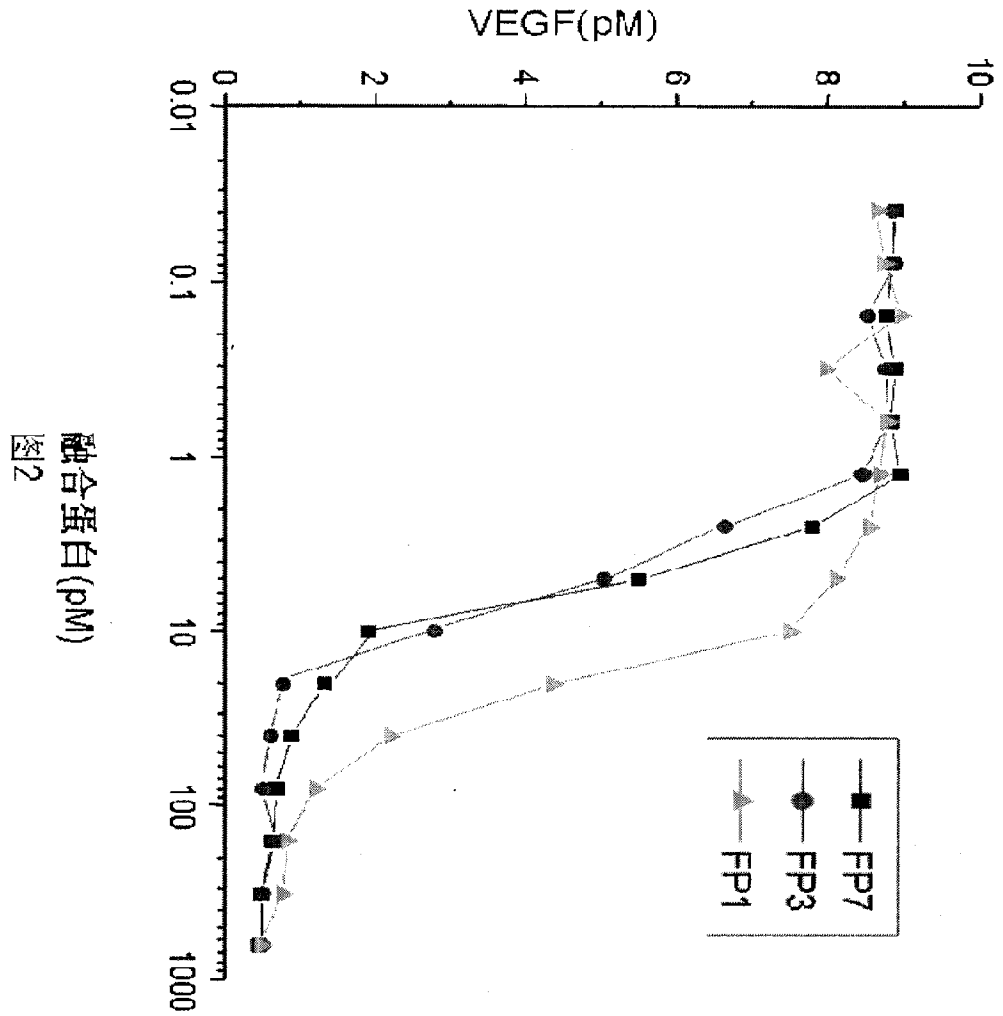


图2

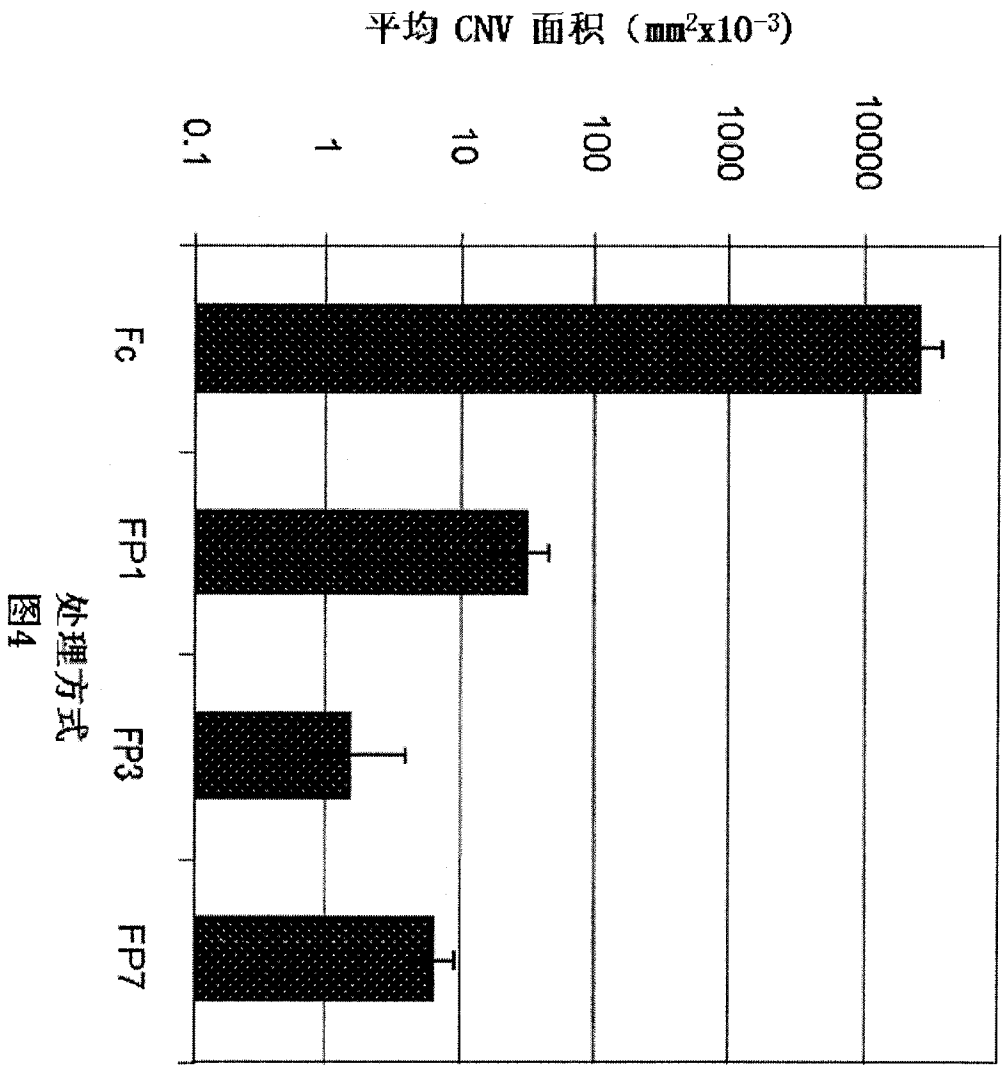


图4

[19] 中华人民共和国国家知识产权局

[12] 发明专利说明书

专利号 ZL 200810160842.8

[51] Int. Cl.
C07K 19/00 (2006.01)
C12N 15/62 (2006.01)
C12N 15/85 (2006.01)
A61K 38/17 (2006.01)
A61K 9/19 (2006.01)
A61K 9/08 (2006.01)

[45] 授权公告日 2009 年 12 月 9 日

[11] 授权公告号 CN 100567325C

[51] Int. Cl. (续)
A61P 27/02 (2006.01)
[22] 申请日 2006.3.31
[21] 申请号 200810160842.8
分案原申请号 200610066257.2
[73] 专利权人 成都康弘生物科技有限公司
地址 610036 四川省成都市金牛区蜀西路 36 号
[72] 发明人 俞德超
[56] 参考文献
CN1222192A 1999.7.7
CN1701814A 2005.11.30
CN1706867A 2005.12.14
审查员 唐 莉

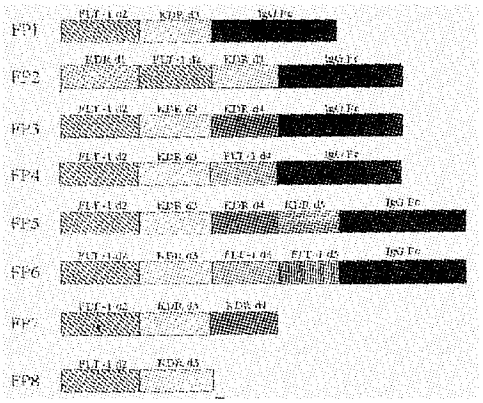
权利要求书 1 页 说明书 11 页 附图 4 页

[54] 发明名称

VEGF 受体融合蛋白及其在制备治疗眼睛疾病的药物中的应用

[57] 摘要

本发明涉及两个 VEGF 受体融合蛋白、其制备方法及其在治疗眼睛疾病中的应用，所述眼睛疾病包括年龄相关性黄斑变性，糖尿病视网膜病变，糖尿病黄斑瘤腺体以及由新生血管生长而引发的治疗失败如激光凝固体，手术视网膜移植。



1.血管内皮生长因子 VEGF 的受体融合蛋白 FP7，其氨基酸序列如序列表中的序列 1 所示。

2.编码血管内皮生长因子 VEGF 受体融合蛋白 FP7 的基因，其核苷酸序列如序列表中的序列 3 所示。

3.含有如权利要求 2 所述的血管内皮生长因子 VEGF 受体融合蛋白 FP7 的基因的表达载体。

4.一种制备如权利要求 1 所述的 VEGF 的受体融合蛋白 FP7 的方法，包括如下步骤：利用基因重组技术构建含有融合蛋白基因的载体，将该载体导入基因工程细胞，表达该融合蛋白。

5.一种药物组合物，包含治疗有效量的权利要求 1 所述的 VEGF 的受体融合蛋白 FP7 以及任选的一种或多种药物学上可接受的载体。

6.根据权利要求 5 所述的药物组合物，所述载体选自 PH3-9 之间的缓冲液、稳定剂、防腐剂、助溶剂、赋形剂、渗透压调节剂中的一种或多种。

7.根据权利要求 6 所述的药物组合物，所述载体选自磷酸盐缓冲液、琥珀酸盐缓冲液、组氨酸缓冲液、甘露醇、海藻糖、聚山梨醇酯、氯化钠、蔗糖、三羟甲基氨基甲烷或乳糖中的一种或多种。

8.根据权利要求 5-7 任一项所述的药物组合物，其制备成适于使用的药物制剂，所述制剂选自冻干制剂或溶液制剂。

9.根据权利要求 8 所述的药物组合物，其制剂的给药方式选自玻璃体内注射、静脉注射给药、腹腔注射、皮下注射或以眼药水滴眼方式给药。

10.权利要求 1 所述的融合蛋白 FP7、权利要求 2 所述的基因或权利要求 5-9 任一项所述的药物组合物在制备治疗与新生血管生长有关的眼科疾病的药物中的应用，其中所述与新生血管生长有关的眼科疾病选自年龄相关黄斑变性，糖尿病视网膜病变，糖尿病性黄斑水肿，视网膜静脉阻塞，由新生血管生长而引发的治疗失败。

11.根据权利要求 10 所述的应用，所述的由新生血管生长而引发的治疗失败选自激光凝固体，手术视网膜移植。

VEGF 受体融合蛋白及其在制备治疗眼睛疾病的药物中的应用

本申请是申请日为 2006 年 3 月 31 日，申请号为 200610066257.2，发明名称为“VEGF 受体融合蛋白在治疗眼睛疾病中的应用”的中国发明专利申请的分案申请。

技术领域：

本发明涉及 VEGF 受体融合蛋白在治疗因新生血管的生长而引发的各种眼科疾病中的应用。

背景技术：

视网膜血管系统和脉络膜(chorodial) 血管系统是组成视网膜的重要组成部分。创伤或疾病而引起的血管管壁结构或功能的异常改变是导致视力下降或丧失的主要原因。例如，糖尿病视网膜病变（diabetic retinopathy）是由于糖尿病而导致视网膜血管增生，进而造成视网膜脱落。它是造成视力丧失的主要原因。在眼睛受伤后或手术后的恢复过程中也可能造成视网膜内新生血管的生长。这一类视网膜血管增生也是造成视力下降或眼睛失明的重要原因（Nature 438: 932-938, 2005）。

年龄相关的黄斑变性（AMD）是一种由视网膜中心区的细胞或组织退化和血管增生而引起的疾病。可分干性和湿性两种。其中湿性 AMD 是脉络膜新生血管形成的最常见形式，它也是引起眼睛失明的主要原因。

血管内皮生长因子(VEGF)是一种专一性调控新生血管生长的蛋白质(Am. J. Pathol. 167:1451-1459, 2005)。VEGF 刺激内皮细胞分裂增生，进而促进新生血管生长，以提供营养和氧气给组织细胞。很多研究表明一旦眼睛视网膜的感光细胞由于营养不足而开始萎缩（称作“缺血性萎缩”），VEGF 在视网膜内的浓度便开始升高，从而促进新生血管生长。这一过程称为“新生血管的形成”（angiogenesis）。在眼内，新生的血管在形态上与正常血管不同，管腔不规则，管壁多为渗漏。这种高通透性或渗漏的血管的异常增生常导致视网膜上产生疤痕，并进一步可发生脱落从而影响到视力。

许多研究表明湿性AMD病人的脉络膜组织中有高水平的VEGF表达(Invest. Opthal. Vis. Sci 37 :855－868,1996 ; Microvascular Res. 64:162－169, 2002)。由于VEGF表达水平与湿性AMD之间的相关性，VEGF可以被用作诊断AMD的一个生化指标（Br. J. Opthalmol. 88:809－815,2004)。

一些VEGF抑制剂可以阻断VEGF与内皮细胞上VEGF受体（flt—1、KDR等）之间的相互作用，从而阻止由VEGF介导的信息传导，抑制由VEGF高表达而引起的新生血管的生长，以达到预防和阻止视网膜上出血的目的。这类VEGF抑制剂包括Macugen (pegaptanib sodium), Lucentis, VEGF-Trap, Avastin (bevacizumab) 和AdPEDF等。其中Macugen和Avastin已由美国国家食品和药物管理局（FDA）批准上市。

发明内容：

本发明提供一类抑制VEGF的融合蛋白，可用于治疗由于新生血管的生长而引发的各种眼科疾病。所述疾病包括（但不限于）所述眼睛疾病包括年龄相关性黄斑变性，糖尿病视网膜病变，糖尿病黄斑瘤腺体以及由新生血管生长而引发的治疗失败如激光凝固体，手术视网膜移植。在本发明中，所述融合蛋白是指大分子化合物，特别是VEGF受体的重组融合蛋白。更具体而言，本发明的VEGF抑制剂是中国专利申请“抑制血管新生的融合蛋白质及其用途”（申请号CN200510073595.4）中描述的FP_1、FP_2、FP_3、FP_4、FP_5、FP_6和本发明提供的FP_7和FP_8中的任何一种。其中FP_1、FP_2、FP_3、FP_4、FP_5、FP_6的融合蛋白氨基酸序列在上述专利说明书中，FP_7和FP_8的氨基酸序列在本发明序列表中的序列1和序列2。

本发明所述的VEGF受体融合蛋白可以通过上述专利说明书中的方法制备，其中FP_1、FP_2、FP_3、FP_4、FP_5、FP_6是已知物，FP_7和FP_8是新物质，对于新物质，本发明提供了其优选的制备方法实施例，其制备原理和上述专利说明书相同。

本发明经过重组技术制成的制品经过纯化达到药用纯度，然后根据制剂的需要再将其制备成药物制剂，这些制剂应该特别适合于静脉注射给药，玻璃体内注射给药，腹腔注射，皮下注射，局部眼内给药，其制备工艺可以采用药物制剂的常规制法完成，优选的是溶液制剂或干粉制剂，作为干粉制剂，使用时将干粉溶解使成为溶液。本发明的融合蛋白制剂，需要时可以加入药物可接受的载体，所述载体可以是任何适合本发明的制剂形式的药物载体，优选的选自：磷酸钠

实施例 1，FP_7的构建

融合蛋白 FP7 是由引物 flt－1 D2(F)、 flt—1 D2 （R)、KDR D3 （F） 和 KDR D3－4 （R）(见专利申请书，申请号 CN200510073595.4)从 HUVEC 细胞提取到的 mRNA 作为模板合成的 cDNA 上放大到的 flt—1 和 KDR 基因片段重组而成。具体的条件是在变性 95° 、30 分钟，退火 56° 、45 秒钟，延伸 72° 、2 分钟的条件下，进行 PCR 扩增，30 个循环，获得 flt—1 和 KDR IgG 样结构域的 PCR 产物。用 TA cloning 试剂盒，把 PCR 产物克隆到 PCR2.1 质粒中，并转染 *E. coli*，选取白色菌落，加入 LB 培养基，培养过夜。用 Qiangen 质粒提取试剂盒提取质粒后酶切及测序鉴定。采用拼接 PCR (Sewing PCR) 方法，把 flt－1 片段、KDR 片段和 IgG 铰链区局部序列的核糖核酸一起连接。在两端引物中设计 EcoR1 酶切位点。PCR 终产物在用 EcoR1 酶切后，经 Qiangen 纯化试剂盒纯化 DNA 片段，并插入 pcDNA3.1 质粒。重组质粒转染 *E. coli*，选取阳性菌落，加入 LB 培养基，培养过夜。Qiangen 质粒提取质粒后酶切，并测序鉴定。已获证实的质粒再转染 CHO 细胞就得稳定表达融合蛋白 FP7 的细胞系。FP_7的具体核甘酸序列见序列表 3。在此融合蛋白 C 末端保留有铰链区 （hinge） 的部分序列。

实施例 2，FP_8的构建

融合蛋白 FP8 是以 FP7 为模板直接用 PCR 扩增而成，PCR 所用引物是 flt-1D2 （F） 和 KDR D3-hing (R) 。后者的序列为：5'-aggtgctgggcacagtgggcatgtgtgagttttgtctttttcatggaccctgacaaatg。它包括与 KDR 第三免疫球蛋白样区相互补的序列和人 IgG Fc 绞链区的部分核苷酸序列。PCR 扩增和基因克隆的方法与实例 1 相同。最终将插入了 FP8 的 Pc DNA3.1 质粒转染 CHO 细胞，并获得稳定细胞株，用于蛋白质的表达。FP8 的氨基酸序列见序列表 2，核苷酸序列见序列表 4。

实施例 3 融合蛋白与 VEGF 结合亲和力的实验

本发明用测定 VEGF 的量来确定各种融合蛋白结合 VEGF 的能力。在这一试验中，将一定量的 VEGF （10PM） 加入试管中，然后将经稀释的含有不同量的各种融合蛋白加到含有 VEGF 的试管中，混合好后，在 37° 的培养箱中保存一个小时。一个小时以后，试管中游离的 VEGF 由 R&D 系统公司 （R&D systems） 提供的检测 VEGF 量的试剂盒——VEGF 检测试剂盒 （VEGF assay Kit） 测定。测定到的结果

（sodium phosphate）、琥珀酸钠（sodium succinate）、 组氨酸（histidine）、甘露醇（mannitol）、海藻糖（trehalose dihydrate）、聚山梨醇酯（polysorbate 20）、氯化钠(sodium chloride)、蔗糖(sucrose)、三羟甲基氨基甲烷(trometamol)或乳糖，上述制剂缓冲液（formulation buffer），应含有 pH 缓冲系统如磷酸盐（phosphate）、柠檬酸盐（citrate）、乙酸盐（acetate）、琥珀酸盐（succinate）、三羟甲基氨基甲烷（trometamol，又名 Tris）或组氨酸（histidine）等中的一种，pH 的范围在 3 至 9；可含有渗透压调节剂如氯化钠（sodium chloride）、葡萄糖（dextrose）等；可含有稳定剂如氨基酸（amino acids）、甘油（glycerol）、环糊精（cyclodextrin）、蔗糖（sucrose）、海藻糖（trehalose dihydrate）等；可含有防腐剂如噻汞撒（thimerosal）、亚硫酸氢钠（sodium bisulfite）、苯基乙醇（benzyl alcohol）等。对于冻干制剂，可含有赋形剂如甘露醇（mannitol）等；对于溶液制剂，可含有表面活性剂如聚山梨醇酯（polysorbate 20 或 80）、十二烷基磺酸钠（SDS）等。融合蛋白的浓度范围在 0.01mg/ml 至 1000mg/ml。其用量根据临床的需要而定。另外制剂中根据需要还可加入防腐剂，稳定剂，助溶剂等辅助成分，可以选自任何适宜的药剂学常规的辅助成分。溶剂选择水或其他等渗溶液，缓冲溶液等。

本发明中，同时还介绍了 VEGF 抑制剂的给药方法。这些抑制剂可以通过多种不同的给药途径给予病人，其中包括（但不限于）静脉给药，玻璃体内注射，也可以通过以点眼药的方式在一定剂型下给予病人以达到治疗眼疾的目的。

本发明意外的发现本发明的融合蛋白形式的 VEGF 抑制剂较现有技术具有优良的眼病治疗作用，而且稳定性好，安全性高，副作用小，效果优良，本发明通过实验数据证明了本发明的有益效果，具体数据见实施例。

附图说明：

图 1：本发明的 8 种融合蛋白的结构示意图

图 2：融合蛋白与 VEGF 结合的亲和力的比较

图 3：融合蛋白对视网膜缺血性萎缩引起的新生血管形成的影响

图 4：融合蛋白对脉络膜新生血管生长的影响

具体实施方式：

以下通过实施例进一步说明本发明，但不作为对本发明的限制。

经软件的处理而得到如图 2 的结果，图 2 表明，FP_1、FP_3和 FP_7都能有效地与 VEGF 亲和结合，其结合亲和能力可由 IC_{50}表示，分别是 11.2PM、4.3PM 和 4.1 PM。这个实验证明，FP_3和 FP_7在体外与 VEGF 的结合能力相似，且两者都高于 FP_1。

这个试验结果进一步说明了 KDR 的第四免疫球蛋白样区域的氨基酸序列可增进融合蛋白对 VEGF 的结合能力。

实施例 4　融合蛋白阻止由视网膜缺血性萎缩引起的新生血管的形成的实验结果

将出生七天的幼鼠放在含高氧分压（75%±2%）的培养箱内，并控制温度为 23℃±2℃，日光照明。在此条件下培养几天之后，视网膜中心将无血管新生发生，五天之后，将幼鼠放回到正常氧分压的培养箱中。 由于室内相对较低的氧分压浓度而对幼鼠的视网膜产生低氧条件，从而刺激产生类似于糖尿病视网膜病变和其他由于缺血性萎缩视网膜病变的新生血管反应。

利用这个模型，可以对三种融合蛋白（FP_1、FP_3和 FP_7）在缺血性萎缩视网膜病变相关的新生血管生成方面的作用做出评估。

将幼鼠放在高氧分压的培养箱中，五天之后带回到正常氧分压室内的培养箱中。幼鼠被分成五个组，每组 10 只，一天之后以 30mg/kg 的量将融合蛋白经腹腔注射幼鼠，每两天注射一次，一共注射 4 次。对照组的幼鼠则被注射含相同量的 Fc 蛋白。治疗期结束后，从各组取小鼠 6 只，心脏注射荧光素 FAM，10 分钟后，幼鼠的视网膜被摘取用来分析新生血管的生成情况。操作时将视网膜放平，在荧光显微镜下观察新生血管及荧光渗漏情况，各组其余四只小鼠的眼睛用石蜡包埋，切片后用 H&E 染色。镜下计数血管内皮细胞核的数目，从而判断融合蛋白对新生血管生长的影响（Investigative Ophthalomogy visual science 43,1994—2000，2002）。结果如图 3 所示。接受 Fc 蛋白注射的幼鼠视网膜都表现出严重的病变。在视网膜表面内脉膜上可观察到大量杂乱无章的血管。在经融合蛋白处理过的幼鼠其视网膜上则无明显的血管生长（如图 3）.其中 FP_3最为有效，FP_7也较有效，但其效果类似于 FP_1，其主要原因可能是因缺失 Fc 片段而影响它在体内的稳定性。FP_1与 FP_7的效果类似。

同时，我们也试验了将这些融合蛋白在通过玻璃体内给药的情况下对新生血管的影响。试验采用同样的动物模型，在动物回到正常氧分压的培养箱内一天后，

以每只眼睛 0.5 毫克的量进行玻璃体内注射，每只动物只接受一次治疗。给药之后第七天，动物的视网膜按上述方法收集和处理。融合蛋白对新生血管的影响如图 3 所示。结果表明，经玻璃体内注射，这些融合蛋白对新生血管的生长具有显著的抑制作用。玻璃体内注射的作用效果要优于腹腔注射。同时，通过这组实验，我们观察到 FP_7 的效果类似于 FP_3 两者都要好于 FP_1。在这个试验中，由于是玻璃体内注射，FP7 不需经体内血液循环。因此，其血液内稳定性较低的物质不会影响它的疗效。

实施例 5　融合蛋白对激光诱发的脉络膜新生血管生长的影响

根据发表的文献资料(American Journal Pathology 153,1641-1646,1998)，我们利用激光在大鼠的眼睛上建立能诱发眼底脉络膜新生血管生成的 AMD 模型。将 150 只左右的大鼠分成四组，对照组的 10 只大鼠通过皮下注射接受 Fc 蛋白（20 mg/kg），处理过的每 10 只大鼠分别通过皮下注射 20 mg/kg 的 FP_1、FP_3 和 FP_7 总共接受 5 次注射，分别在激光处理前一天和激光处理之后的第 3、6、9 和 12 天。在激光处理后第 15 天，大鼠通过静脉注射接受 50mg 荧光标记的右旋糖苷，然后经麻醉处理，将眼睛摘除，尽快剥离脉络膜，剖成扁平状或冷冻包埋做切片用于分析 CNV 病变情况。结果如图 4 表明，经融合蛋白处理小鼠的 CNV 面积都比对照（Fc）要小，其中 FP_3 的效果要优于 FP_1 和 FP_7。FP_7 与 FP_1 的抑制 CNV 生成方面效果相当。

实施例 6　融合蛋白在治疗眼疾中的应用

这些融合蛋白可以通过适当的方式，如玻璃体内注射或静脉给药，可用于治疗与病变新生血管生长相关的一系列眼科疾病，其中包括年龄相关性黄斑变性（AMD)、糖尿病视网膜病变(diabetic retinopathy)、糖尿病黄斑水肿(diabetic macular edema)和视网膜血管阻塞 (central retinal vein occlusion)。同时这些融合蛋白也可以和其他治疗方法一起结合使用，如和光敏药物（photocoagulation）或者与激光疗法结合使用，以降低在激光处理之后由新生血管生长引起的治疗失败机率。这些融合蛋白还可以和手术结合使用。如在视网膜移植之后，由于新生血管的生长而使视网膜移植的手术失败。如果在手术的同时，病人接受这些融合蛋白的治疗，就可以提高视网膜移植的成功率。AMD 病人在通过正常眼科检查之后，建立基本的基准线，然后通过玻璃体注射的方法将融

合蛋白（如 FP_3 或 FP_7）注入体内。经处理后病人将到医院接受观察和检查，以记录这些融合蛋白对 AMD 的影响。一般在接受治疗之后第 1、2、6、14、30 和 90 天分别检查一次。同时，病人可能需要接受多次治疗，可以是每两到八周注射一次。每次注射的量为每只眼睛 10 微克至 5 毫克范围内。

实施例 7　FP3 融合蛋白冻干制剂的制备

先配制好制剂缓冲液（formulation buffer），将合格的原液（drug substance）解冻后，用制剂缓冲液稀释到所需的蛋白浓度。进行过滤除菌后，用移液器/分装器按要求装量分装至洁净的西林瓶（规格：0.5ml/2ml）中，往瓶口加洁净的丁基橡胶塞（半压塞）。放入冷冻干燥机中，设定好合适的冻干曲线（包括预冻、冷冻、抽真空、升温各阶段的时间、温度、真空度等参数的设定），进行冻干。当冻干过程结束后，压紧胶塞，取出西林瓶，在胶塞上加铝塑盖，用轧盖器轧紧。往西林瓶上贴标签，装入纸盒，存放于适宜的温度。

实施例 8　FP3 融合蛋白溶液制剂的制备

先配制好制剂缓冲液（formulation buffer），将合格的原液（drug substance）解冻后，用制剂缓冲液稀释到所需的蛋白浓度。进行过滤除菌后，用移液器/分装器按要求装量分装至洁净的西林瓶（规格：5ml/20ml）中，往瓶口加洁净的丁基橡胶塞，塞紧。在丁基橡胶塞上加铝塑盖，用轧盖器轧紧。往西林瓶上贴标签，装入纸盒，存放于适宜的温度。

实施例 9　FP3 融合蛋白眼用制剂的制备

先配制好制剂缓冲液，将合格的原液（drug substance）解冻后，用制剂缓冲液稀释到所需的蛋白浓度。进行过滤除菌后，用移液器按要求装量（≤200μl）分装至洁净的西林瓶（规格：0.5ml）中，或用玻璃注射器（规格：1ml，带灰橡胶活塞、27 号针头）抽吸至要求装量（≤100μl）。对于西林瓶，在瓶口端加洁净的丁基橡胶塞，塞紧，在丁基橡胶塞上加铝塑盖，用轧盖器轧紧，往西林瓶上贴标签；对于注射器，在活塞处加装橡胶塞，在针头上加装灰橡胶罩，在橡胶罩外再加装硬塑套，用铝封袋（已印好标签）封装（另配有带螺纹的塑料活塞杆、白色法兰盘接头，用不同的铝封袋封装）。装入纸盒，存放于适宜的温度。

实施例 10　FP1 融合蛋白眼用制剂的制备，制备方法同实施例 9

实施例 11　FP2 融合蛋白眼用制剂的制备，制备方法同实施例 9

实施例 12 FP4 融合蛋白眼用制剂的制备，制备方法同实施例 9

实施例 13 FP5 融合蛋白眼用制剂的制备，制备方法同实施例 9

实施例 14 FP6 融合蛋白眼用制剂的制备，制备方法同实施例 9

实施例 15 FP7 融合蛋白眼用制剂的制备，制备方法同实施例 9

ThrCysProLeuCysProAlaPro 308

<210>2

<211>214

<212>PRT

<213>人工序列

<400>2

GlyArgProPheValGluMetTyrSerGluIleProGluIleIle 15

HisMetThrGluGlyArgGluLeuValIleProCysArgValThr 30

SerProAsnIleThrValThrLeuLysLysPheProLeuAspThr 45

LeuIleProAspGlyLysArgIleIleTrpAspSerArgLysGly 60

PheIleIleSerAsnAlaThrTyrLysGluIleGlyLeuLeuThr 75

CysGluAlaThrValAsnGlyHisLeuTyrLysThrAsnTyrLeu 90

ThrHisArgGlnThrAsnThrIleIleAspValValLeuSerPro 105

SerHisGlyIleGluLeuSerValGlyGluLysLeuValLeuAsn 120

CysThrAlaArgThrGluLeuAsnValGlyIleAspPheAsnTrp 135

GluTyrProSerSerLysHisGlnHisLysLysLeuValAsnArg 150

AspLeuLysThrGlnSerGlySerGluMetLysLysPheLeuSer 165

ThrLeuThrIleAspGlyValThrArgSerAspGlnGlyLeuTyr 180

ThrCysAlaAlaSerSerGlyLeuMetThrLysLysAsnSerThr 195

PheValArgValHisGluLysAspLysThrHisThrCysProLeu 210

CysProAlaPro 214

<210>3

<211>924

<212>DNA

<213>人工序列

<400>3

ggtagacctt tcgtagagat gtacagtgaa atccccgaaa ttatacacat gactgaagga 60

agggagctcg tcattccctg ccgggttacg tcacctaaca tcactgttac tttaaaaaag 120

tttccacttg acactttgat ccctgatgga aaacgcataa tctgggacag tagaaagggc 180

序列表

<110>成都康弘生物科技有限公司

<120> VEGF 受体融合蛋白在制备治疗眼睛疾病的药物中的应用

<160>4

<210>1

<211>308

<212>PRT

<213>人工序列

<400>1

GlyArgProPheValGluMetTyrSerGluIleProGluIleIle 15

HisMetThrGluGlyArgGluLeuValIleProCysArgValThr 30

SerProAsnIleThrValThrLeuLysLysPheProLeuAspThr 45

LeuIleProAspGlyLysArgIleIleTrpAspSerArgLysGly 60

PheIleIleSerAsnAlaThrTyrLysGluIleGlyLeuLeuThr 75

CysGluAlaThrValAsnGlyHisLeuTyrLysThrAsnTyrLeu 90

ThrHisArgGlnThrAsnThrIleIleAspValValLeuSerPro 105

SerHisGlyIleGluLeuSerValGlyGluLysLeuValLeuAsn 120

CysThrAlaArgThrGluLeuAsnValGlyIleAspPheAsnTrp 135

GluTyrProSerSerLysHisGlnHisLysLysLeuValAsnArg 150

AspLeuLysThrGlnSerGlySerGluMetLysLysPheLeuSer 165

ThrLeuThrIleAspGlyValThrArgSerAspGlnGlyLeuTyr 180

ThrCysAlaAlaSerSerGlyLeuMetThrLysLysAsnSerThr 195

PheValArgValHisGluLysProPheValAlaPheGlySerGly 210

MetGluSerLeuValGluAlaThrValGlyGluArgValArgIle 225

ProAlaLysTyrLeuGlyTyrProProProGluIleLysTrpTyr 240

LysAsnGlyIleProLeuGluSerAsnHisThrIleLysAlaGly 255

HisValLeuThrIleMetGluValSerGluArgAspThrGlyAsn 270

TyrThrValIleLeuThrAsnProIleSerLysGluLysGlnSer 285

HisValValSerLeuValValTyrValProProAspLysThrHis 300

ttcatcatat caaatgcaac gtacaaagaa atagggcttc tgacctgtga agcaacagtc 240

aatgggcatt tgtataagac aaactatctc acacatcgac aaaccaatac aatcatagat 300

gtggttctga gtccgtctca tggaattgaa ctatctgttg gagaaaagct tgtcttaaat 360

tgtacagcaa gaactgaact aaatgtgggg attgacttca actgggaata cccttcttcg 420

aagcatcagc ataagaaact tgtaaaccga gacctaaaaa cccagtctgg gagtgagatg 480

aagaaatttt tgagcacctt aactatagat ggtgtaaccc ggagtgacca aggattgtac 540

acctgtgcag catccagtgg gctgatgacc aagaagaaca gcacatttgt cagggtccat 600

gaaaaacctt ttgttgcttt tggaagtggc atggaatctc tggtggaagc cacggtgggg 660

gagcgtgtca gaatccctgc gaagtacctt ggttacccac ccccagaaat aaaatggtat 720

aaaaatggaa taccccttga gtccaatcac acaattaaag cggggcatgt actgacgatt 780

atggaagtga gtgaaagaga cacaggaaat tacactgtca tccttaccaa tcccatttca 840

aaggagaagc agagccatgt ggtctctctg gttgtgtatg tcccaccgga caaaactcac 900

acatgcccac tgtgcccagc acct 924

<210>4

<211>642

<212>DNA

<213>人工序列

<400>4

ggtagacctt tcgtagagat gtacagtgaa atccccgaaa ttatacacat gactgaagga 60

agggagctcg tcattccctg ccgggttacg tcacctaaca tcactgttac tttaaaaaag 120

tttccacttg acactttgat ccctgatgga aaacgcataa tctgggacag tagaaagggc 180

ttcatcatat caaatgcaac gtacaaagaa atagggcttc tgacctgtga agcaacagtc 240

aatgggcatt tgtataagac aaactatctc acacatcgac aaaccaatac aatcatagat 300

gtggttctga gtccgtctca tggaattgaa ctatctgttg gagaaaagct tgtcttaaat 360

tgtacagcaa gaactgaact aaatgtgggg attgacttca actgggaata cccttcttcg 420

aagcatcagc ataagaaact tgtaaaccga gacctaaaaa cccagtctgg gagtgagatg 480

aagaaatttt tgagcacctt aactatagat ggtgtaaccc ggagtgacca aggattgtac 540

acctgtgcag catccagtgg gctgatgacc aagaagaaca gcacatttgt cagggtccat 600

gaaaaagaca aaactcacaca tgcccactgt gcccagcac ct 642

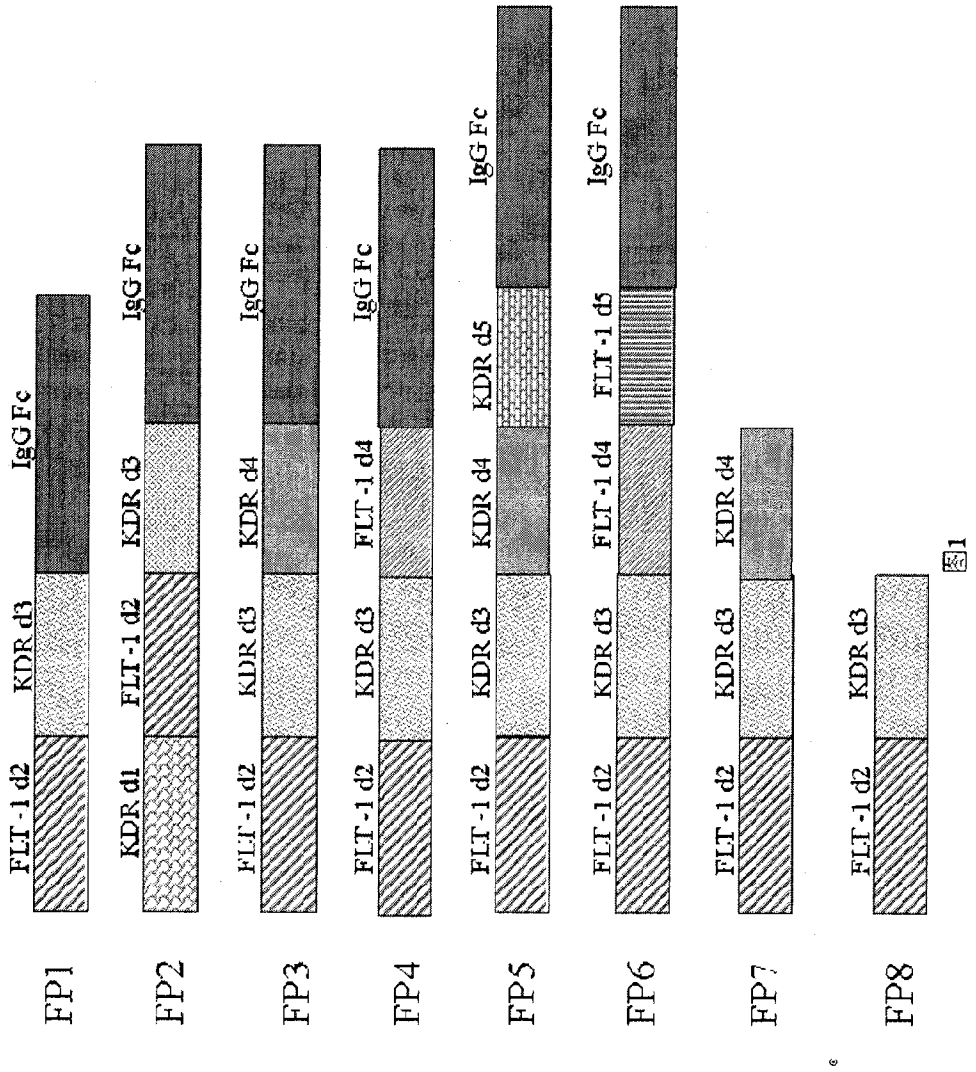


图1

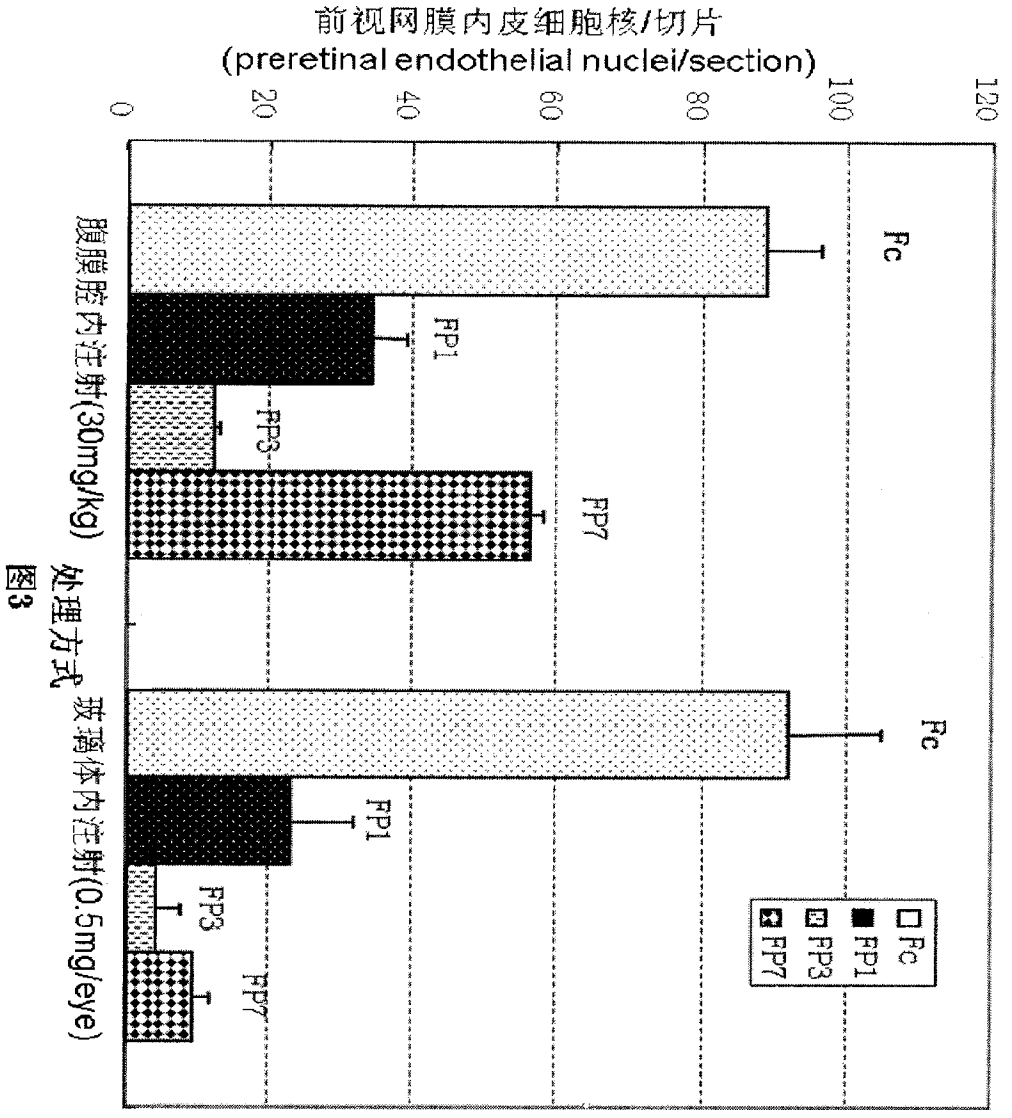


图3

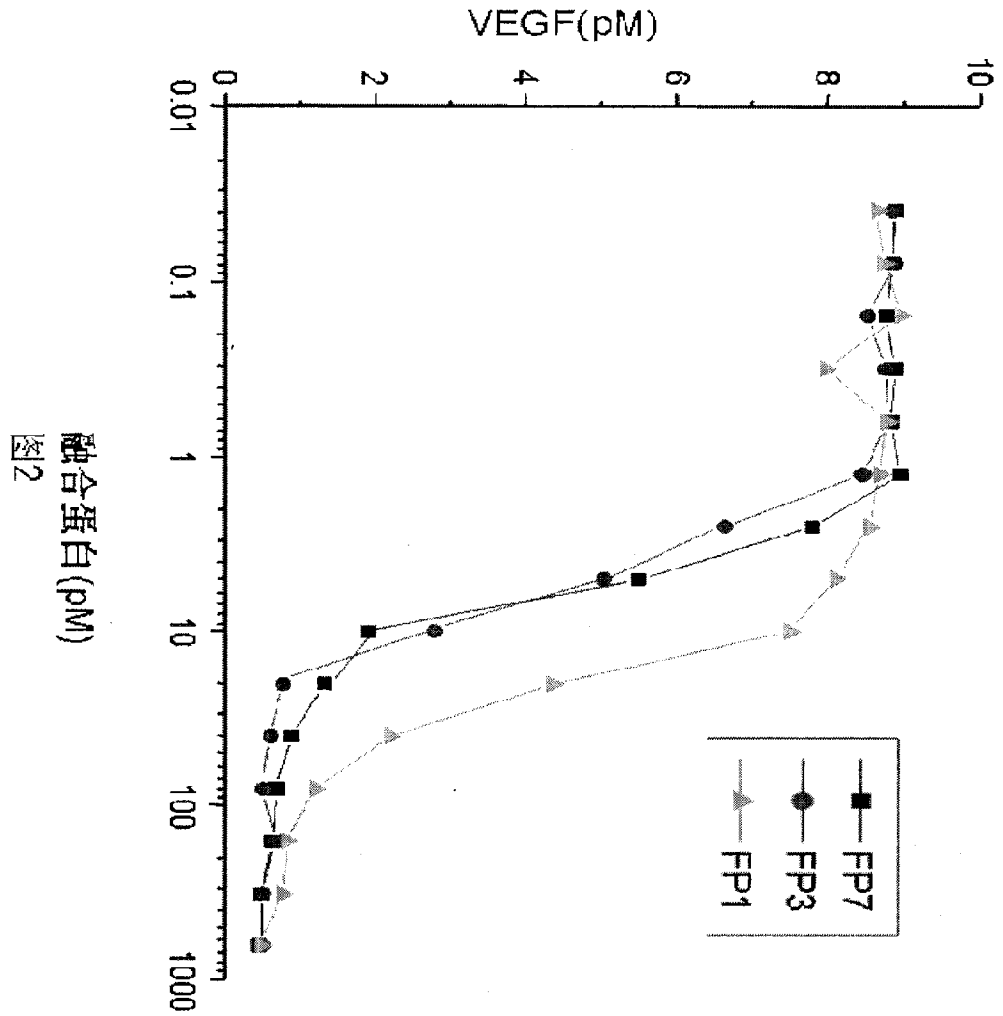


图2

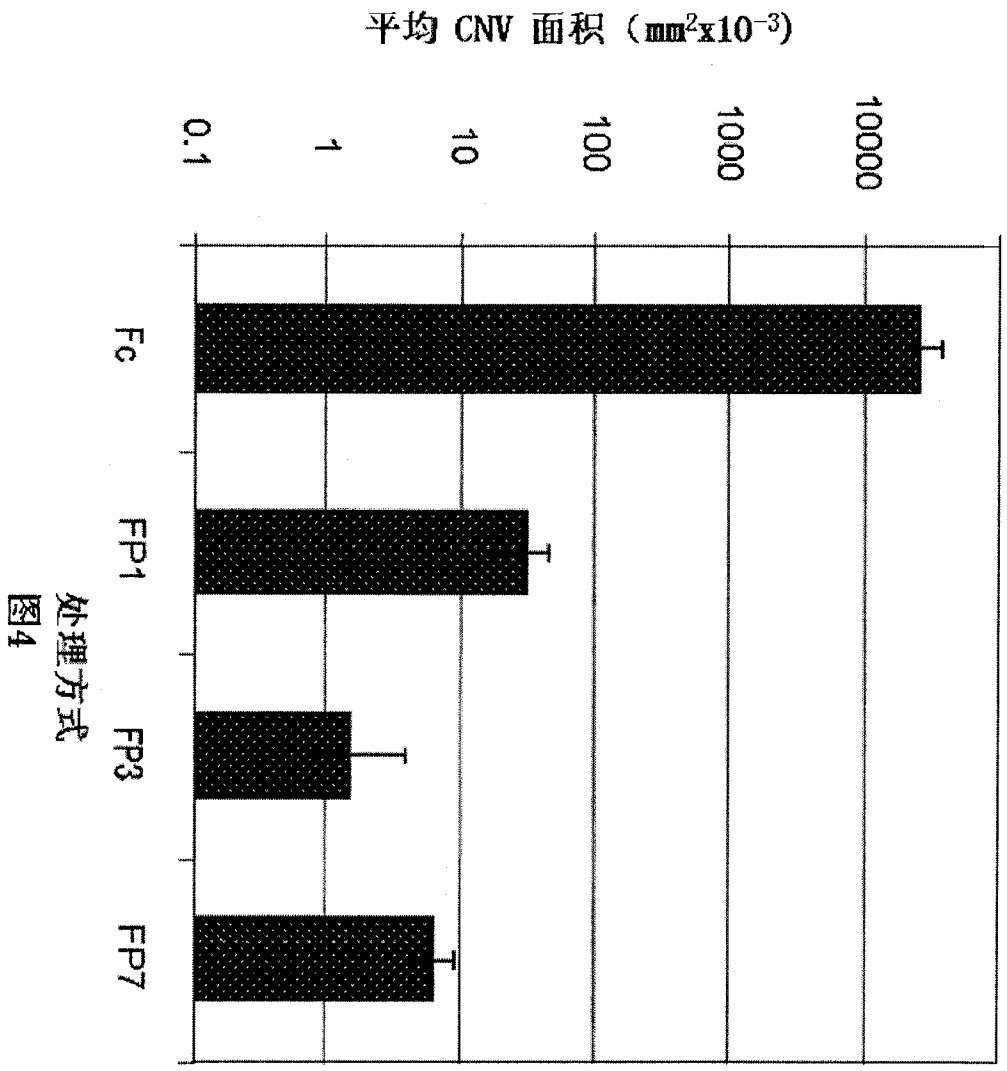


图4

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 July 2012 (19.07.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/097019 A1

(51) International Patent Classification:
A61K 38/18 (2006.01) *A61P 27/00* (2006.01)

(21) International Application Number:
PCT/US2012/020855

(22) International Filing Date:
11 January 2012 (11.01.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/432,245	13 January 2011 (13.01.2011)	US
61/434,836	21 January 2011 (21.01.2011)	US
61/561,957	21 November 2011 (21.11.2011)	US

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

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **YANCOPOULOS, George D.** [US/US]; 1519 Baptist Church Road, Yorktown Heights, NY 10598 (US).

(74) Agent: **COTTINGHAM, Frank**; Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, 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, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, 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, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

---- *with international search report (Art. 21(3))*

---- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

---- *with sequence listing part of description (Rule 5.2(a))*

(54) **Title**: USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

(57) **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 and corneal neovascularization.

WO 2012/097019 A1

USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

FIELD OF THE INVENTION

[0001] 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

[0002] 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.

[0003] 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.

[0004] 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

[0005] 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.).

[0006] 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.

[0007] 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."

[0008] 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).

[0009] 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.

[0010] Other embodiments of the present invention will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURE

[0011] 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

[0012] 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.

[0013] 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.).

[0014] 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

[0015] 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.

[0016] 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.

[0017] 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.

[0018] 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.

[0019] 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.

[0020] 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

[0021] 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.

[0022] 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").

[0023] 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.

[0024] 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

[0025] 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 choroidal neovascularization, age-related macular degeneration (AMD), diabetic retinopathies, diabetic macular edema (DME), central retinal vein occlusion (CRVO), corneal neovascularization, and retinal neovascularization.

PHARMACEUTICAL FORMULATIONS

[0026] 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.

[0027] 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

[0028] 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

[0029] 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.

[0030] 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 deteciable 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.

[0031] 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

[0032] 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

[0033] 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.

[0034] 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

[0035] 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

[0036] 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

[0037] 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.

[0038] 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

[0039] 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.

[0040] 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.

[0041] 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.

[0042] 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

[0043] 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).

[0044] 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.

[0045] 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.

[0046] Approximately 1200 subjects were enrolled, with a target enrollment of 300 subjects per treatment arm.

[0047] 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.

[0048] 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.

[0049] 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).

[0050] 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.

[0051] 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.

[0052] The study procedures are summarized as follows:

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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.

[0057] 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.

[0058]

C. Results Summary (52 Week Data)

[0059] 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

[0060] 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.

[0061] 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)

[0062] 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 weeks[a] (2Q8)	42	8.5**	9.7**
VEGFT 2 mg as needed[a] (PRN)	45	10.3**	12.0**

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

[0063] 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

[0064] 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

[0065] 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.

[0066] 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 6.6% 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.

[0067] 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.

SEQUENCES

[0068] SEQ ID NO:1 (DNA sequence having 1377 nucleotides):

ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGTCTGCTTCTC
ACAGGATCTAGTTCCGGAAGTGATACCGGTAGACCTTTCGTAGAGATGTACAGTGAAATCC
CCGAAATTATACACATGACTGAAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCAC
CTAACATCACTGTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGC
ATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGC
TTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAAACTATCTCACACATCGA
CAAACCAATACAATCATAGATGTGGTTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTGG
AGAAAAGCTTGTCTTAAATTGTACAGCAAGAACTGAACTAAATGTGGGGATTGACTTCAACT
GGGAATACCCTTCTTCGAAGCATCAGCATAAGAAACTTGTAAACCGAGACCTAAAAACCCA
GTCTGGGAGTGAGATGAAGAAATTTTTGAGCACCTTAACTATAGATGGTGTAACCCGGAGT
GACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACA
TTTGTCAGGGTCCATGAAAAGGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAA
CTCCTGGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATC
TCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGT
CAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG
AGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT

GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC
CATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCT
ATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAG
ACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG
GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG
CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

[0069] SEQ ID NO:2 (polypeptide sequence having 458 amino acids):

MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITV
TLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLS
PSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLT
IDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

[0070] 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 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.

21. A VEGF antagonist for use in a method of treating an angiogenic eye disorder in a patient, wherein the method comprises 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.

22. The VEGF antagonist 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. The VEGF antagonist 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. The VEGF antagonist of any one of claims 21 to 23, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

25. The VEGF antagonist of any one of claims 21 to 23, 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. The VEGF antagonist of any one of claims 21 to 25, 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 and corneal neovascularization.

27. The VEGF antagonist of claim 26, wherein the angiogenic eye disorder is age related macular degeneration.

28. The VEGF antagonist of any one of claims 21 to 27, 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.

29. The VEGF antagonist of claim 28, wherein the VEGF antagonist is a VEGF receptor-based chimeric molecule.

30. The VEGF antagonist of claim 29, wherein the VEGF receptor-based chimeric molecule comprises VEGFR1R2-FcΔC1(a) encoded by the nucleic acid sequence of SEQ ID NO:1.

31. The VEGF antagonist of claim 29, 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.

32. The VEGF antagonist of any one of claims 21 to 31, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

33. The VEGF antagonist of claim 32, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

34. The VEGF antagonist of claim 33, wherein the intraocular administration is intravitreal administration.

35. The VEGF antagonist of claim 34, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

36. The VEGF antagonist of claim 35, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

37. The VEGF antagonist of claim 35, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

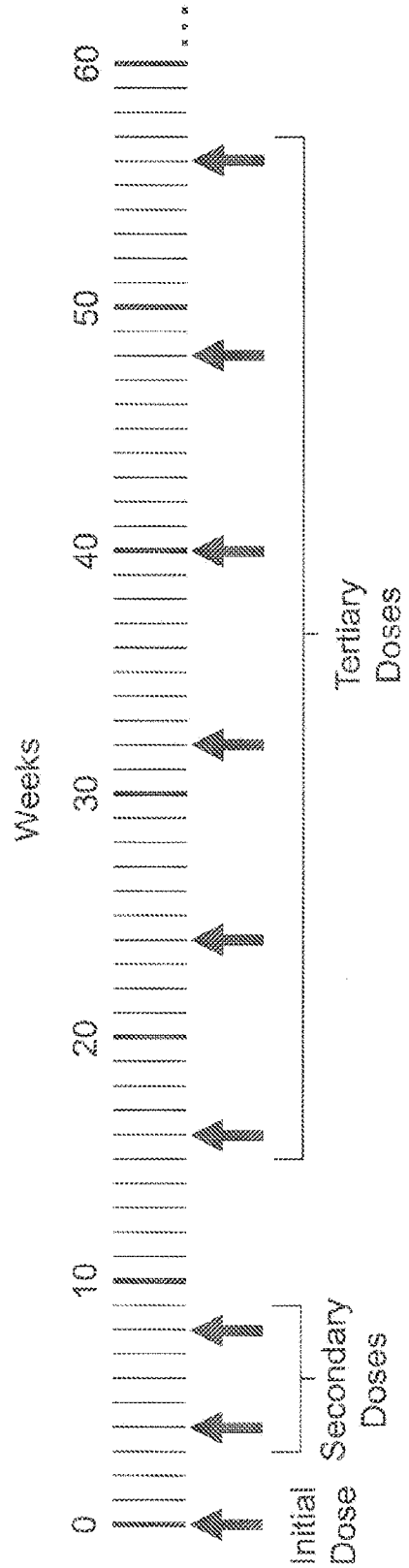

Weeks
0
10
20
30
40
50
60
...
Initial Dose
Secondary Doses
Tertiary Doses

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/020855

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/18 A61P27/00
ADD.

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 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 practicable, search terms used)
EPO-Internal, 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	Thomson Reuters Integrity: "VEGF Trap-Eye final phase II results in age-related macular degeneration presented at 2008 Retina Society Meeting", , 28 September 2008 (2008-09-28), pages 1-1, XP002674126, Retrieved from the Internet: URL:https://integrity.thomson-pharma.com/integrity/xmlxsl/pk_ref_list.xml_show_llist at_refs?p_session_id=1868065&p_orig=&p_count=354&p_num_dailys=42&p_qry_save=&p_subtitol=Biomedical%20Literature%20List&p_dailys=N&p_tsearch=A&p_text=N&p_whatToShow=REF&p_prousQuantity=5&p_page=10#link [retrieved on 2012-04-18] the whole document ----- -/--	1-37

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier application or patent 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
19 April 2012	22/05/2012

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, Fax: (+31-70) 340-3016	Rodrigo-Simón, Ana

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NGUYEN Q D ET AL: "A Phase I Study of Intravitreal Vascular Endothelial Growth Factor Trap-Eye in Patients with Neovascular Age-Related Macular Degeneration", OPHTHALMOLOGY, J. B. LIPPINCOTT CO., PHILADELPHIA, PA, US, vol. 116, no. 11, 1 November 2009 (2009-11-01), pages 2141-2148.E1, XP026732998, ISSN: 0161-6420, DOI: 10.1016/J.OPHTHA.2009.04.030 [retrieved on 2009-08-22] abstract page 2142, column 1 - paragraph 3 page 2147, column 1, paragraph 2	1-37
A	US 2007/190058 A1 (SHAMS NAVEED [US]) 16 August 2007 (2007-08-16) claim 1 paragraph [0112] page 12, paragraph 111	1-37
A	US 2006/172944 A1 (WIEGAND STANLEY J [US] ET AL) 3 August 2006 (2006-08-03) paragraphs [0008], [0017] claims 1,2	1-37
A	US 2005/163798 A1 (PAPADOPOULOS NICHOLAS J [US] ET AL) 28 July 2005 (2005-07-28) claim 65 paragraph [0122]	1-37
A	WO 00/75319 A1 (REGENERON PHARMA [US]; PAPADOPOULOS NICHOLAS J [US]; DAVIS SAMUEL [US]) 14 December 2000 (2000-12-14) page 87, line 14 - line 88 page 14, line 7 - line 12	1-37
A	US 2006/058234 A1 (DALY THOMAS J [US] ET AL) 16 March 2006 (2006-03-16) paragraph [0067] claim 23 page 2, paragraph 15	1-37
A	US 2005/260203 A1 (WIEGAND STANLEY J [US] ET AL) 24 November 2005 (2005-11-24) page 7; example 8 page 8; example 9 page 8; example 11 page 9; example 2	1-37

-/--

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DO DV 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 OPHTHALMOL, vol. 93, no. 2, February 2009 (2009-02), pages 144-1449, XP009158490, abstract page 149, paragraph 2	1-37
A	ANANT PAI ET AL.: "Current concepts in intravitreal drug therapy for diabetic retinopathy", SAUDI JOURNAL OF OPHTHALMOLOGY, vol. 24, no. 4, 30 June 2010 (2010-06-30), pages 143-149, XP002674122, page 147, paragraph 4	1-37
X,P	DO DIANA V ET AL: "The DA VINCI Study: phase 2 primary results of VEGF Trap-Eye in patients with diabetic macular edema.", OPHTHALMOLOGY SEP 2011 LNKD-PUBMED:21546089, vol. 118, no. 9, September 2011 (2011-09), pages 1819-1826, XP002674123, ISSN: 1549-4713 the whole document	1-37
T	STEWART MW.: "The expanding role of vascular endothelial growth factor inhibitors in ophthalmology", MAYO CLIN PROC, vol. 87, no. 1, January 2012 (2012-01), pages 77-88, XP002674124, the whole document	
A	anonymous: "LUCENTIS® (ranibizumab injection) Intravitreal Injection", , June 2006 (2006-06), pages 1-3, XP002674125, Retrieved from the Internet: URL:http://www.gene.com/gene/products/information/pdf/lucentis-prescribing.pdf [retrieved on 2012-04-18] page 1, column 1, line 20 - page 1, column 2, line 9	1-37

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/020855

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2007190058 A1	16-08-2007	NONE	
US 2006172944 A1	03-08-2006	US 2006172944 A1	03-08-2006
		US 2008085276 A1	10-04-2008
US 2005163798 A1	28-07-2005	US 7070959 B1	04-07-2006
		US 2005163798 A1	28-07-2005
		US 2005175610 A1	11-08-2005
		US 2005245447 A1	03-11-2005
		US 2008194460 A1	14-08-2008
		US 2008194799 A1	14-08-2008
		US 2008220004 A1	11-09-2008
		US 2009081217 A1	26-03-2009
		US 2009155899 A1	18-06-2009
		US 2010221782 A1	02-09-2010
		US 2011028698 A1	03-02-2011
		US 2012064621 A1	15-03-2012
WO 0075319 A1	14-12-2000	AT 293164 T	15-04-2005
		AT 417928 T	15-01-2009
		AU 779303 B2	13-01-2005
		AU 5040400 A	28-12-2000
		BR 0011407 A	02-04-2002
		CA 2376379 A1	14-12-2000
		CN 1369009 A	11-09-2002
		CN 101433715 A	20-05-2009
		CZ 20014387 A3	16-10-2002
		DE 60019415 D1	19-05-2005
		DE 60019415 T2	09-03-2006
		DK 1544299 T3	23-03-2009
		EP 1183353 A1	06-03-2002
		EP 1544299 A1	22-06-2005
		ES 2237429 T3	01-08-2005
		ES 2319305 T3	06-05-2009
		HK 1043388 A1	10-06-2005
		HR 20010908 A2	30-04-2005
		HU 0201515 A2	28-08-2002
		IL 146890 A	08-07-2008
		JP 4723140 B2	13-07-2011
		JP 2003501089 A	14-01-2003
		JP 2011024595 A	10-02-2011
		MX PA01012630 A	22-07-2002
		NO 20016036 A	08-02-2002
		NZ 515913 A	30-01-2004
		PL 352246 A1	11-08-2003
		PT 1183353 E	30-06-2005
		PT 1544299 E	18-03-2009
		SK 17522001 A3	01-04-2003
		WO 0075319 A1	14-12-2000
		ZA 200110068 A	06-12-2002
US 2006058234 A1	16-03-2006	NONE	
US 2005260203 A1	24-11-2005	NONE	

Notice

ABSTRACT CN102233132B

[0001]

13 The present invention relates to the application of VEGFR fusion proteins FP1, FP3, FP7, and FP8 in the preparation of drugs for the treatment of ocular surface neovascularization related diseases, and the combined preparation of fusion proteins FP1, FP3, FP7 and FP8 and immunosuppressive agents. The immunosuppressive agent is selected from any one or a combination of corticosteroids, rapamycin, dexamethasone, and cyclosporine A, wherein the amino acid sequences of FP1, FP3, FP7 and FP8 are as shown in SEQ ID NO:1 and 2 respectively , 3 and 4.

CN102233132B Application of VEGF acceptor fusion proteins in preparation of drugs for inhibiting growth of ocular surface neovascularization

Technical field

The present invention relates to the medical application of Vascular Endothelial Growth Factor (VEGFR) fusion protein, in particular to the application of VEGFR fusion protein in the preparation of medicine for treating diseases caused by the growth of ocular surface neovascularization.

Background technique

The cornea and conjunctiva on the surface of the eye are susceptible to various stimuli, including microbial infections, physical impact, chemical damage, corneal friction damage caused by contact lenses, corneal hypoxia, etc., can cause corneal or conjunctival lesions, these diseases often produce eyes Table neovascularization. Diseases related to ocular surface neovascularization include corneal neovascularization, neovascular glaucoma, pterygium, conjunctivitis, bacterial keratitis, fungal keratitis, viral keratitis, vesicular keratoconjunctivitis, corneal ulcer, scleritis , Complications of contact lens, etc.

Corneal neovascular diseases include corneal trauma (including puncture wounds, rupture wounds, scratches, insect bites, chemical burns, physical burns, etc.), corneal transplant rejection, ocular surface damage caused by anterior and posterior segment surgery Corneal stromal ulcer scar formation, foreign body stimulation, and contact lens-related hypoxia lead to corneal neovascularization, limbal stem cell deficiency, malnutrition and vitamin deficiency, adverse drug reactions, and iatrogenic injury.

The cornea is one of the important refractive stroma of the eyeball, which is transparent and avascular under normal conditions. However, under the pathological conditions of infection, injury, hypoxia and corneal transplantation, the capillaries grow from the limbus to the cornea. , Forming corneal neovascularization. Although the occurrence of corneal neovascularization is conducive to the local clearing of infection and the promotion of damage repair (Curr Opin Ophthalmol. 12: 242-249, 2001), the occurrence of corneal neovascularization can cause changes in the immune characteristics of corneal tissue (ExpEyeRes.78 : 579-589, 2004), eventually leading to clinical problems such as vision loss, corneal damage and loss of ocular surface shielding function. Corneal neovascularization often leads to persistent inflammation and scarring of tissues. Corneal neovascularization is the main feature in many blinding keratopathy lesions, and it is also a complication of corneal infection, chemical injury and major visual impairment after corneal transplantation. At the same time, neovascularization also affects the success rate of corneal transplantation.

At present, eye diseases caused by corneal neovascularization in developed countries account for the top cause of blindness, and the blindness rate reaches 57.2%. Corneal neovascularization caused by ocular trauma in China accounts for about 10% of the entire corneal disease. Therefore, the prevention and treatment of corneal neovascularization has become an urgent problem to be solved.

Although laser treatment and surgical intervention provide potential treatment for corneal neovascularization, some problems remain after treatment. For example, in most cases, the avascular and transparent state of the cornea of patients undergoing laser treatment and surgical intervention cannot be reconstructed. In addition, the drugs currently used to treat corneal neovascularization are mainly hormonal drugs such as dexamethasone, which have the defects of expanding the corneal injury area after treatment, slow corneal healing, and more side effects. Therefore, it is very important to provide a safe and effective therapeutic drug for the prevention and early treatment of ocular surface neovascularization and ocular surface inflammation.

The occurrence of corneal neovascularization is a complex pathological process. The results of studies have confirmed that the main cause of corneal neovascularization is due to the inclination of the balance between angiogenic factors and anti-angiogenic factors in ocular surface tissues. Diseases can secondaryly express cytokines that promote vascular growth and induce ocular surface development in a direction conducive to

corneal neovascularization. Among them, vascular endothelial growth factor is the most important vascular endothelial growth factor found so far (Science246 : 1306-9, 1989).

Chinese patent ZL200510073595.4 discloses the structure and preparation method of fusion protein FP1-FP6 and its anti-tumor use, as well as for visual mesh lesions, but does not involve the prevention and treatment of corneal neovascularization. Chinese patent ZL200610066257.2 discloses the structure of fusion protein FP1-FP8 and its preparation method, as well as its application in the preparation of drugs for the treatment of ophthalmic diseases caused by the growth of neovascularization, also does not involve VEGFR fusion proteins FP1, FP3 FP7 and FP8 are used in the preparation of drugs for treating corneal neovascularization.

Neovascular glaucoma refers to glaucoma caused by the formation of new fibrous vascular membranes on the surface of the iris and trabecula, which prevents the drainage of aqueous humor. Its neovascularization is easy to rupture and recurrent hemorrhage of the anterior chamber. . This kind of disease is a serious disease, easy to relapse, and more stubborn secondary glaucoma. At present, general anti-glaucoma drugs and surgical treatments are often difficult to work together. Although laser or cryosurgery is often used, it is easy to relapse and even lead to blindness. . The patient's eyes were hyperemia, corneal edema, severe eye pain and headache. In order to relieve the pain, some patients had to remove the eyeballs.

The cause of pterygium is still unknown, and it may be related to ultraviolet radiation and smoke pollution. In recent years, studies have suggested that the disease is related to neovascularization and is also related to allergic reactions and elastic fibrosis. At present, there is no good treatment for this kind of disease. Although it can be treated by surgery, it has a greater impact on patients, and safe and effective prevention and treatment drugs are urgently needed.

Conjunctivitis is a common and frequent disease in ophthalmology. Among them, antibiotics or hormones are mainly used for the prevention and treatment of acute conjunctivitis. Chronic conjunctivitis is easy to relapse and difficult to cure, resulting in dry eyes and foreign body sensation in patients. Long-term illness will cause vision loss, vascular hyperplasia, and also involve the cornea, causing keratitis and corneal ulcers. The effective prevention and treatment of these diseases also urgently need safe and effective prevention and treatment drugs.

The technical contents disclosed in Chinese patents ZL200510073595.4 and ZL200610066257.2 are used as reference for this application.

Summary of the invention

An object of the present invention is to provide a VEGFR fusion protein FP1, FP3, FP7, FP8 for the preparation of drugs for treating ocular surface neovascularization-related disorders, wherein the amino acid sequences of FP1, FP3, FP7 and FP8 are as SEQ ID NO: 1, 2, 3, 4 shown.

In a preferred embodiment of the present invention, the ocular surface neovascularization-related disorders are selected from corneal neovascularization, conjunctival neovascularization, conjunctivitis, pterygium, neovascular glaucoma, keratitis, bacterial keratitis, fungal Keratitis, viral keratitis, corneal ulcer, alveolar keratoconjunctivitis, scleritis, contact lens complications, or any complications thereof, preferably corneal neovascularization, conjunctival neovascularization, or their complications disease.

In one embodiment of the present invention, the corneal neovascularization-related disorders are selected from corneal trauma, corneal transplant rejection, ocular surface damage caused by anterior and posterior segment surgery, corneal stromal ulcer scar formation, foreign body stimulation, and hypoxia Corneal neovascularization, and any of or complications of neovascular disease caused by limbal stem cell deficiency, malnutrition, vitamin deficiency, adverse drug reactions, or iatrogenic injury.

In a preferred embodiment of the present invention, the corneal trauma is selected from any one of puncture wounds, rupture wounds, scratches, insect bites, chemical burns, physical burns or complications of the ocular surface.

In a preferred embodiment of the present invention, the chemical burn is selected from acid burn or alkali burn.

In a preferred embodiment of the present invention, the viral keratitis is selected from viral keratitis caused by herpes simplex virus (HSV) infection.

In a preferred embodiment of the present invention, the corneal neovascularization-related disorders are selected from herpes simplex virus infection, surgical sutures, corneal transplantation, acid burn or alkali burn, any one of which induces or causes corneal neovascularization .

The VEGFR fusion proteins FP1, FP3, FP7 and FP8 of the present invention are blocked by capturing multiple subclasses of the VEGF family (such as VEGF-A, VEGF-B, VEGF-C, VEGF-D) and placental growth factor (PIGF) Inhibit the activation of its receptor, thereby showing the effect of blocking or inhibiting the biological activity of VEGF protein.

The VEGFR fusion proteins FP1, FP3, FP7 and FP8 of the present invention are all made of human VEGFR functional domain proteins and / or human IgG FC fragment proteins. Specifically, FP1 consists of the second immunoglobulin-like region of VEGFR1 and the third immunoglobulin-like region of VEGFR2; FP3 consists of the second immunoglobulin-like region of VEGFR1, the third and fourth immunoglobulin-like regions of VEGFR2 Region, and the FC segment protein of human IgG immunoglobulin; FP7 is composed of the second immunoglobulin-like region of VEGFR1, the third and fourth immunoglobulin-like regions of VEGFR2, and the FC segment protein of human IgG immunoglobulin FP8 is composed of the second immunoglobulin-like region of VEGFR1, the third immunoglobulin-like region of VEGFR2, and the FC segment protein of human IgG immunoglobulin.

The method provided by the invention can also be used for treating acute and subacute corneal injury. The acute injury is within 1 week after injury. The clinical manifestations are oedema and thrombosis of the ocular surface tissues, as well as degeneration and necrosis of the affected cornea, conjunctiva and vascular endothelial cells. The subacute phase is generally within 2 weeks to 6 months after injury. The clinical manifestations are the proliferation and migration of various cells on the ocular surface, repair of damage and defects, and the appearance of corneal epithelial cells replaced by conjunctival epithelium, and a large number of corneal neovascularization) Formation, a large number of leukocytes invade under the damaged tissue. In various specific applications, the treatment of acute injury should be carried out within 24 hours after injury, and the treatment of subacute injury should be based on the local comprehensive situation of the ocular surface, and the optimal treatment time should be selected.

Another object of the present invention is to provide a combination preparation of VEGFR fusion proteins FP1, FP3, FP7, and FP8 with an immunosuppressant selected from the group consisting of corticosteroids, rapamycin, dexamethasone, and cyclosporin A Any one or a combination thereof, preferably dexamethasone.

The combined preparation of the present invention can be administered sequentially or sequentially, that is to say, when the medical staff administers to the patient, they can first use the VEGFR fusion protein FP1, FP3, FP7 and FP8 preparation for the patient, and then use it for the patient The immunosuppressive agent according to the present invention; or the immunosuppressive agent according to the present invention to the patient first, and then the VEGFR fusion protein FP1, FP3, FP7 and FP8 preparation to the patient; , FP7 and FP8 preparations and the immunosuppressive agents described in this invention.

BRIEF DESCRIPTION

Figure 1 Effect of fusion proteins FP1, FP3, FP7 and FP8 on corneal neovascularization induced by alkali burn in rats

Figure 2 Inhibitory effects of fusion proteins FP1, FP3, FP7 and FP8 on mouse corneal angiogenesis induced by surgical suture

Fig 3 Inhibitory effect of fusion proteins FP1, FP3, FP7 and FP8 on mouse corneal lymphangiogenesis induced by surgical suture

Figure 4 Inhibitory effect of fusion protein FP3 on rabbit corneal angiogenesis induced by alkali burn

Figure 5 Inhibitory effect of fusion proteins FP1, FP3 and immunosuppressants on corneal angiogenesis after corneal transplantation in rats

detailed description

The following examples further illustrate the present invention, but should not be construed as constituting any limitation to the present invention.

Example 1 The therapeutic effect and safety test of fusion protein on corneal angiogenesis induced by alkali burn

An animal model of corneal angiogenesis in rats (SD rats, female, body weight 250g ± 20g) was established by alkali burn method, and the inhibitory effect of fusion proteins FP1, FP3, FP7, and FP8 on rat corneal angiogenesis induced by alkali burn was studied .

Take the rat and use the right eye as the experimental eye under anesthesia, use a filter paper with a diameter of 3mm soaked with 1mol / L NaOH solution and stick it to the center of the right eye cornea of the rat. After 1 minute, remove the filter paper and use a large amount Wash the conjunctival sac with physiological saline to remove residual NaOH, and add chloramphenicol eye drops (manufacturer: Changzhou Siyao Pharmaceutical Co., Ltd., specifications 8ml: 20mg) 20ul and 1% atropine ointment.

The next day, the rats were divided into 7 groups (6 rats in each group), namely FP1 group, FP3 group, FP7 group, FP8 group, drug carrier group (ie PBS solution), dexamethasone group and saline group (NS). Among them, the FP1, FP3, FP7, and FP8 groups were all administered with fusion protein at a concentration of 20 mg / ml. They were prepared with PBS solution immediately before use. Sodium (Na2HPO4), 8.0g sodium chloride (NaCl), 0.2g potassium chloride (KCl), add water to 1000mL, add NaOH to adjust pH7.4; the concentration of dexamethasone group is 0.25mg / ml. The solution of each group was added to the eye surface of each group of animals 4 times a day, 50 microliters each time, the administration time was 8:00, 12:00, 16:00, 20:00, for 14 consecutive days. At the same time, 15 days after the model was made, the corneal fluorescence staining photography was performed to observe the effect of the drug on the ulcer area formed after alkali burn of the cornea, and the safety of the drug was evaluated.

On the 15th day after alkali burn, the animals were anesthetized, corneal neovascularization was stained with ink, the images were observed under the microscope and images were collected, and corneal neovascularization was calculated according to Robert formula A = C / 12 × 3.1416 [R2- (RL) 2] The growth area, where A represents the area of neovascularization, C is the number of minutes of the new cornea's cumulative corneal circumference, L is the length of the neovascularization extending from the limbus into the cornea, and R is the average corneal radius of the test animal (2.6mm in this experiment)) , 结果 见表 1。 The results are shown in Table 1.

Table 1 Inhibitory effect of fusion protein on corneal neovascularization after alkali burn

Corneal neovascularization area (mm ⟨sup⟩ 2 ⟨/ sup⟩) FP1 treatment group 7.4 ± 1.5 FP3 treatment group 3.0 ± 0.7 FP7 treatment group 4.2 ± 1.1 FP8 treatment group 4.1 ± 1.3 dexamethasone group 9.8 ± 1.0 drug carrier Group 10.2 ± 1.6 physiological saline group 11.0 ± 1.2

The results in Table 1 show that compared with the dexamethasone group, the VEGFR fusion proteins FP1, FP3, FP7, and FP8 can significantly inhibit corneal angiogenesis, and can be used to treat ocular surface burn diseases, and the fusion protein FP3 has an effect on ocular surface angiogenesis The inhibition ability is relatively stronger, and the inhibition on the length and area of corneal neovascularization in rats is more significant. In addition, the corneal edema of the animals in the fusion protein treatment group was lighter, the neovascularization was smaller and sparsely distributed, mostly located near the limbus, and the blood vessels did not enter the central corneal burn area, and the ratio of the length and area of corneal neovascularization in the FP3 group It was significantly smaller than other treatment groups, drug carrier group and saline group (p (0.05), and also significantly better than dexamethasone group.

Table 2 Effect of fusion protein on corneal injury after alkali burn

Corneal damage area after 14 days of medication (mm ⟨sup⟩ 2 ⟨/ sup⟩) FP1 treatment group 2.5 ± 0.4 FP3 treatment group 2.0 ± 0.9 FP7 treatment group 2.4 ± 1.3 FP8 treatment group 2.7 ± 1.2 dexamethasone group 9.4 ± 1.4 Drug carrier group 2.3 ± 1.2 physiological saline group 2.1 ± 1.1

In the dexamethasone treatment group, after 14 days of continuous medication, although corneal neovascularization was suppressed, corneal injury was aggravated and the area of the injury was ulcerated.

However, the corneal injury in the fusion protein treatment group did not increase, which was comparable to the saline control group, and it had good safety.

It can be seen that the fusion protein group inhibits neovascularization and has no side effects of dexamethasone to inhibit corneal healing, and the efficacy and safety are better than dexamethasone.

Example 2 The therapeutic effect and safety of fusion protein on herpes simplex virus keratitis

An animal model of corneal injury infected with herpes simplex virus in mice (BalB / C mice, female, 6-8 weeks old, body weight 20-25 g) was prepared using the corneal scratch method.

Herpes simplex virus was subcultured on vero cells and quantified by conventional methods. The right eye of the mouse is the experimental eye. Under anesthesia, use a micro-dose needle dipped in 2 μl of HSV-1 virus liquid (about 5 × 105 PFU herpes simplex type 1 virus) to gently scratch the animal cornea and Massage the conjunctiva.

The experimental animals were divided into 7 groups (8 per group), namely FP1 group, FP3 group, FP7 group, FP8 group, drug carrier group, dexamethasone group and saline group (NS). Among them, the FP1, FP3, FP7, and FP8 groups were all administered with a fusion protein concentration of 10 mg / ml, and were diluted with PBS solution before use; the drug carrier group was a PBS solution; the dexamethasone group was administered a concentration of 0.25 mg / ml. The solution of each group was added to the eye surface of each group 4 times a day, 10 μl each time. The administration time was 8:00, 12:00, 16:00, 20:00, and the administration was continued for 14 days.

After the treatment, the animals were anesthetized, and slit lamps were used to observe the degree of angiogenesis and damage on the ocular surface. The corneal injury of each group of animals was evaluated by the injury index, and the specific injury index was divided according to the following criteria: 0: normal cornea; 1: slight turbidity; 2: moderate turbidity; 3: severe turbidity (invisible iris); 4: severe opacity With ulcers; 5: corneal rupture. The distribution of the number of animals in each group of animals at each injury level is shown in Table 3. Among them, a small damage index indicates that the damage to the ocular surface is smaller, and the smaller the number of animals with a smaller damage index, the smaller the damage to the cornea and the better the protection.

The results showed that the rats with damage index ≤2 in FP1 group, FP3 group, FP7 group, and FP8 group were 50%, 75%, 62.5%, and 50%, respectively, and 25% in drug carrier group and normal saline group , 25% in the dexamethasone group, indicating that FP3 has a better treatment for viral keratitis and does not expand corneal damage, and has a better protective effect.

Table 3 Animal statistics of corneal injury index

Injury index ≤1 Injury index = 2 Injury index = 3 Injury index = 3 Injury index = 4 25% 25% 25% 25% 25% FP3 37.5% 37.5% 12.5% 12.5% 12.5% FP7 25% 15% 25% 25% % FP8 treatment group 25% 25% 12.5% 37.5% Dexamethasone group 12.5% 12.5% 12.5% Drug carrier group 12.5% 12.5% 25% 50% Saline group 25% 12.5%

The degree of corneal angiogenesis is evaluated by the angiogenesis index. The specific angiogenesis index is mainly based on the range of neovascular invasion of the cornea. A 16-point system is used, which divides the cornea into 16 zones (4 quadrants of the cornea and 4 zones within each quadrant) , 1 point is scored by blood vessel invasion, and the final angiogenesis index is the sum of the number of blood vessel invasion corneal regions.

Observations were made on the 7th and 15th days of administration respectively, and the angiogenesis index of corneal neovascularization in each group of animals was calculated. Compared with the drug carrier group and saline group, the growth trend of corneal angiogenesis index of FP1 group, FP3 group, FP7 group and FP8 group was significantly reduced (p ⟨0.05), and the FP3 protein had the strongest and significantly better action For the dexamethasone group, the results are shown in Figure 1.

It can be seen that the fusion proteins FP1, FP3, FP7, and FP8 can significantly inhibit corneal angiogenesis and reduce the degree of corneal damage, and can be used to treat viral keratitis with safety.

Example 3 Inhibition of fusion protein on mouse corneal angiogenesis induced by surgical suture

An animal model of corneal angiogenesis was established by implanting surgical sutures in the corneal stroma of mice.

Take 6-week-old male BalB / c mice, weighing 18-25g, under anesthesia, using the right eye as the experimental eye, implant 3 surgical sutures (11-0 nylon thread) in the corneal stroma (each The line starts near the limbus and ends at the central cornea, and the angle between two adjacent lines is 120 °).

Three days after planting sutures, the animals were divided into the following 7 groups (6 animals per group), namely FP1 group, FP3 group, FP7 group, FP8 group, drug carrier group, dexamethasone group, and saline group (NS). Among them, the FP1, FP3, FP7, and FP8 groups were all administered with a fusion protein concentration of 10 mg / ml, and were diluted with PBS solution before use; the drug carrier group was a PBS solution; the dexamethasone group was administered a concentration of 0.25 mg / ml. The solution of each group was added to the eye surface of each group of animals 4 times a day, 50 microliters each time, the administration time was 8:00, 12:00, 16:00, 20:00, for 14 consecutive days.

After the treatment, the animals were sacrificed and corneal tissue was taken for immunohistochemical staining. FITC (fluorescein isothiocyanate) labeled CD31 (platelet endothelial cell adhesion molecule-1) showed neovascularization, and Cy3 (indocyanine green dye) labeled LYVE-1 (lymphatic endothelial cell hyaluronic acid receptor) Body-1) shows neonatal lymphatic vessels. Under a fluorescence microscope, the cornea is divided into 8 zones, and the fluorescence images (100 ×) of each zone are collected in sequence. The area of neovascularization and lymphatic vessels is calculated using image analysis software. The corneal neovascularization or lymphatic vessel area of mice in the saline group was 100%. The growth of corneal neovascularization or lymphatic vessels in each group of animals was analyzed and expressed as the ratio of neovascularization or lymphatic vessel area.

The results show that the application of eye drops containing fusion protein FP3 through the ocular surface can significantly reduce corneal vascular and lymphangiogenesis induced by sutures. In addition, the corneal neovascularization (p (0.05) and neoplastic lymphatic vessel area ratio (p (0.05) of the FP3 group were significantly smaller than those of the control group, as shown in Figure 2 and Figure 3.

It can be seen that the fusion proteins FP1, FP3, FP7, and FP8 can obviously inhibit the regeneration of corneal blood vessels and lymphatic vessels, and can be used to treat traumatic ocular surface neovascular diseases, ocular surface inflammation, and keratitis caused by contact lens.

Example 4 Inhibitory effect of subconjunctival injection of fusion protein FP3 on rabbit corneal angiogenesis induced by alkali burn

As in Example 2, an animal model of rabbit corneal angiogenesis was established using the alkali burn method.

Take 18 New Zealand white rabbits, male or female, weighing 2.0-2.5kg. Under anesthesia, using the right eye as the experimental eye, apply a filter paper sheet (diameter 5 mm) saturated with NaOH (concentration: 1 mol / L) to the center of the cornea of the right eye of the rabbit. After 1 minute, remove the filter paper sheet and use 10 ml Wash the conjunctival sac with physiological saline to remove residual NaOH, and add chloramphenicol eye drops (manufacturer: Changzhou Siyao Pharmaceutical Co., Ltd., specification 8ml: 20mg) 20ul.

The model animals were randomly divided into the following 3 groups (6 animals in each group), that is, the first day of FP3 administration group, subconjunctival injection of FP3 on the first day after alkali burn; FP3 administration group on the 14th day, the 14th after alkali burn FP3 was injected subconjunctivally in the day; the drug carrier PBS solution control group, and the drug carrier was injected on the first day after alkali burn. The administration volume of the FP3 treatment group was 0.05 ml, and the administration concentration was 10 mg / ml. The administration volume of the control group was the same as that of the treatment group.

21After the day, the animals were anesthetized, the cornea of the rabbit's eye was observed with a microscope, and the area of corneal neovascularization was measured, and the degree of angiogenesis was represented by the percentage of the area of angiogenesis in the entire ocular surface.

It can be seen that the percentage of corneal neovascularization area of the animals in the first day of FP3 administration group is significantly smaller than that in the control group (p (0.01) and the day 14 of FP3

administration group (p (0.05), indicating that FP3 can significantly inhibit the cornea caused by alkali burn Angiogenesis, and early use has a better inhibitory effect on injured angiogenesis.

Example 5 Inhibitory effect of fusion protein on corneal neovascularization caused by acid burn in rats

The corneal neovascularization model of rats (SD rats, female, body weight 200-250g) was established by acid burn method.

Use the same sample pipette tip with an inner diameter of 1.5mm containing absorbent cotton core, dip it into a mixture of 750g / L silver nitrate and 250g / L potassium nitrate, and place it on the cornea of the rat (randomly burn the right or left cornea)) 10s in the center, remove the pipette tip, immediately rinse with saline for 1min.

The next day, the model rats (weight 180g ± 20g) were randomly divided into the following 6 groups (6 in each group), namely FP1 group, FP3 group, FP7 group, drug carrier group, dexamethasone group and saline group (NS). Among them, the concentration of the fusion protein in the FP1, FP3, and FP7 groups is 10 mg / ml, which is diluted with PBS solution before use; the drug carrier group is the PBS solution; the concentration of the dexamethasone group is 0.25 mg / ml . The solution of each group was added to the eye surface of each group 4 times a day, 20 μl each time. The administration time was 8:00, 12:00, 16:00, 20:00, and the administration was continued for 14 days.

15 days after surgery, after intraperitoneal anesthesia with ketamine (60 mg / kg) and chlorpromazine (30 mg / kg), images were collected under a slit lamp microscope to measure the length and number of corneal neovascularization.

Corneal neovascularization area is calculated according to Robert's formula A = C / 12 × 3.1416 [R2- (RL) 2], where A represents the area of neovascularization, C is the number of circumferential minutes of the cornea accumulated by neovascularization, and L is the neovascularization from the limbus The length of the cornea, R is the average radius of the cornea of the test animal (3mm in this experiment).

The results showed that: 15 days after surgery, the corneal edema of the animals in the fusion protein treatment group was lighter, and the neovascularization was smaller and sparsely distributed, mostly located near the upper and lower corneal limbus. In addition, compared with the control group, the length and area of corneal neovascularization in the FP1 group, FP3 group, and FP7 group were significantly reduced (p (0.05). The results are shown in Table 4.

It can be seen that the fusion proteins FP1, FP3, and FP7 have a significant inhibitory effect on inflammatory corneal neovascularization caused by acid burns, and can be used to treat inflammatory diseases of the ocular surface, and FP3 has a better inhibitory effect on angiogenesis of the ocular surface inflammatory disorders.

Table 4 Inhibitory effect of fusion protein on corneal neovascularization after corneal acid burn

Corneal neovascularization area (mm ⟨sup⟩ 2 ⟨/ sup⟩) FP1 treatment group 6.7 ± 1.5 FP3 treatment group 3.0 ± 0.5 FP7 treatment group 4.3 ± 0.6 dexamethasone group 8.8 ± 1.1 drug carrier group 10.9 ± 1.5 Group 11.2 ± 1.6.

Example 6 The therapeutic effect of fusion protein on conjunctivitis

Animal model of rabbit conjunctivitis was prepared by ethanol induction method.

Forty-two Japanese white-eared rabbits were selected, both male and female, weighing 1.8-2.2kg. Randomly divided into 7 groups, 6 in each group, namely FP1 group, FP3 group, FP7 group, FP8 group, drug carrier PBS solution group, dexamethasone group and saline group (NS).

Pull the lower eyelid of the left eye of the rabbit into a cup shape, and use a micro sampler to drip medical grade absolute ethanol into the conjunctival sac. Add 0.135ml on the first day, 0.09ml on the second day, and drop on the third day. 0.045ml, the right eye served as its own control. On the fourth day of observation, conjunctival hyperemia, edema, closed eyes shimmer, increased secretions, and ciliary hyperemia were considered successful modeling.

After successful modeling, each group of drugs was administered. Among them, the concentration of the fusion protein in the FP1, FP3, FP7, and FP8 groups were all 10 mg / ml. They were diluted with PBS solution before use; the drug carrier group was PBS solution; The dosing concentration of the mason group was 0.25 mg / ml. Each group of solutions was added dropwise to the eye surface of each group of animals 4 times a day, 50 μl each time, the administration time was 8:00, 12:00, 16:00, 20:00, continuous application 7 days.

After the treatment, observe the animal conjunctiva and ocular surface. The severity of conjunctivitis in each group of animals is divided according to the following criteria: 1. Conjunctival hyperemia: no hyperemia (-); mild hyperemia (+); obvious hyperemia, deep red (++); diffuse hyperemia, purple-red (+++); 2. Eyelid edema: no edema (-); mild edema (+); obvious hyperemia, partial eyelid valgus (++); obvious edema, eyelid semi-closed (+++); 3. secretion Substances: No abnormal secretions (-); a small amount of secretions (+); secretions make the eyelid or eyelashes wet or sticky (++); secretions make the entire eye area wet or sticky (+++), the results are shown in Table 5 .

Table 5 Effect of fusion protein on rabbit conjunctivitis

The results showed that both the fusion protein treatment group and the dexamethasone group could significantly improve the symptoms of conjunctivitis after the end of medication, and FP3 had the best effect on conjunctivitis.

Example 7 Inhibitory effect of fusion protein on corneal angiogenesis after corneal transplantation in rats

The rat corneal transplantation model was used to study the inhibitory effect of fusion protein on angiogenesis after corneal transplantation.

48 Wistar female rats (weight 180g ± 20g) were used as donors, 96 SD female rats (weight 180g ± 20g) were used as recipients, and the rats were 2-3 months old. The right eye of the recipient animal received corneal transplantation, and the donor eyes were sacrificed after taking materials from both eyes. The donor provides cornea of both eyes, and the recipient chooses right eye surgery. The donor and recipient were dilated with 1% atropine eyedrops and 0.5% tropineamide eyedrops 20 minutes before surgery, 10% chloral hydrate was injected with 3ml / kg intraperitoneal anesthesia, and the eyes were locally anesthetized with topical anesthetics. A 3.5 mm diameter implant was drilled from the donor cornea, placed in a Petri dish and protected with viscoelastic agent. In the right eye of the recipient, a 3.0 mm diameter drill was used to drill the recipient implant bed, and 8 needles were intermittently sutured with non-damaged sutures between the implants. Wire knots are exposed and not buried. Then, the anterior chamber is injected with a balance solution to form the anterior chamber. After the operation, mydriasis was injected, 0.1 ml of gentamicin was injected subconjunctivally, and erythromycin ointment was applied to the conjunctival sac. One stitch of eyelid was sutured with silk thread and removed on the first day after surgery.

The next day, the model recipient rats were randomly divided into 12 groups (8 rats in each group), namely FP3 group, FP1 group, dexamethasone group, cyclosporine A group, rapamycin group, dexamethasone plus FP3 group, cyclosporine A plus FP3 group, rapamycin plus FP3 group, dexamethasone plus FP1 group, cyclosporine A plus FP1 group, rapamycin plus FP1 group, drug carrier PBS solution group. Among them, the administration concentration of the fusion protein in the FP1 and FP3 groups is 10 mg / ml, which is diluted with PBS solution before use; the drug carrier group is the PBS solution; the administration concentration of the dexamethasone group is 0.25 mg / ml; The administration concentration of sporin A was 1 mg / ml; the administration concentration of rapamycin was 1 mg / ml, both of which were administered to the ocular surface. Among them, the FP3 group, FP1 group, dexamethasone group, cyclosporine A group, rapamycin group, and drug carrier group were administered 4 times a day, 20ul each time, and the administration time was 8:00 and 12: 00, 16:00, 20:00, continuous application for 14 days; dexamethasone plus FP3 group, cyclosporine A plus FP3 group, rapamycin plus FP3 group, dexamethasone plus FP1 group, cyclosporine A plus The FP1 group and the rapamycin plus FP1 group are administered sequentially or successively. The FP1 and FP3 protein are administered once a day in the morning and evening, 20ul each time, and the chemical drugs are administered once a day, 20ul each time, continuous application 14 days.

After intraperitoneal anesthesia with ketamine (60 mg / kg) and chlorpromazine (30 mg / kg) 15 days after surgery, images were collected under a slit lamp microscope to measure the length and number of corneal neovascularization.

Corneal neovascularization area is calculated according to Robert's formula A = C / 12 × 3.1416 [R2- (RL) 2], where A represents the area of neovascularization, C is the number of circumferential minutes of the cornea accumulated by neovascularization, and L is the neovascularization from the limbus The length of the cornea, R is the average corneal radius of the test animal (3mm in this experiment), the results are shown in Figure 5.

The results showed that the fusion proteins FP1, FP3 and immunosuppressive agents (rapamycin, dexamethasone, cyclosporin A) had a synergistic effect on corneal angiogenesis inhibition (p (0.05) 15 days after surgery, and The combination of FP3, FP3 and immunosuppressants has the strongest inhibitory effect on corneal angiogenesis. Corneal edema is mild, and neovascularization is small and sparsely distributed.

Sequence Listing

(110) Chengdu Kanghong Biological Technology Co., Ltd.

(120) Application of VEGF receptor fusion protein in the preparation of drugs for treating ocular surface angiogenesis diseases

(130) None

(160)4

(170) PatentInversion3.5

(210)1

(211)455

(212) PRT

(213) Artificial sequence

(400)1

Met Val Ser Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser

1 5 10 15

Cys, Leu, Leu, Leu, Thr, Gly, Ser, Ser, Ser, Gly, Gly, Arg, Pro, Phe, Val, Glu

20 25 30

Met Tyr Ser Ser Glu Ile Pro Glu Ile Ile His His Met Thr Glu Gly Arg Glu

35 40 45

LeuValIleProCysArgArgValThrThrProAsnAsleIleThrThrValThreu

50 55 60

Lys, Lys, Phe, Pro, Leu, Asp, Thr, Leu, Ile, Pro, Asp, Gly, Lys, Arg, Ile, Ile

65 70 75 80

Trp Asp Ser Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu

85 90 95

Ile, Gly, Leu, Leu, Thr, Cys, Glu, Ala, Thr, Val, Asn, Gly, His, Leu, Tyr, Lys

100 105 110

Thr, Asn, Tyr, Leu, Thr, His, Arg, Gln, Thr, Asn, Thr, Ile, Ile, Asp, Val

115 120 125

Leu, Ser, Pro, Ser, His, Gly, Ile, Glu, Leu, Ser, Val, Gly, Glu, Lys, Leu, Val

130 135 140

Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn

145 150 155 160

TrpGluGluTyrProSerSerSysLysHisGlnHisLysLysLysLeuValAsAsArg

165 170 175

Asp, Leu, Lys, Thr, Gln, Ser, Gly, Ser, Glu, Met, Lys, Lys, Phe, Leu, Ser, Thr

180 185 190

Leu Thr Ile Asp Gly Val Thr Arg Ser Ser Asp Gln Gly Leu Tyr Thr Cys

195 200 205

Ala, Ala, Ser, Ser, Gly, Leu, Met, Thr, Lys, Lys, Asn, Ser, Thr, Phe, Val

210 215 220

Val, His, Glu, Lys, Asp, Lys, Thr, His, Thr, Cys, Pro, Leu, Cys, Pro, Ala, Pro

225 230 235 240

Glu, Leu, Leu, Gly, Gly, Pro, Ser, Val, Phe, Leu, Phe, Pro, Pro, Lys, Pro, Lys

245 250 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Val Thys Cys Val Val Val

260 265 270

Asp, Val, Ser, His, Glu, Asp, Pro, Glu, Val, Lys, Phe, Asn, Trp, Tyr, Val, Asp

275 280 285

GlyVal, Glu, Val, His, Asn, Ala, Lys, Thr, Lys, Pro, Arg, Glu, Glu, Gln, Tyr

290 295 300

Asn, Ser, Thr, Tyr, Arg, Val, Ser, Val, Leu, Thr, Val, Leu, His, Gln, Asp

305 310 315 320

TrpLeuAsnGlyLysGluGluTyrLysCysLysValSerAsnLysAlaLeu

325 330 335

Pro, Ala, Pro, Ile, Glu, Lys, Thr, Ile, Ser, Lys, Ala, Lys, Gly, Gln, Pro, Arg

340 345 350

Glu, Pro, Gln, Val, Tyr, Thr, Leu, Pro, Pro, Ser, Arg, Asp, Glu, Leu, Thr, Lys

355 360 365

Asn, Gln, Val, Ser, Leu, Thr, Cys, Leu, Val, Lys, Gly, Phe, Tyr, Pro, Ser, Asp

370 375 380

Ile, Ala, Val, Glu, Trp, Glu, Ser, Asn, Gly, Gln, Pro, Glu, Asn, Asn, Tyr, Lys

385 390 395 400

Ala Thr Pro Pro Val Val Leu Asp Ser Ser Asp Gly Ser Ser Phe Phe Leu Tyr Ser

405 410 415

Lys, Leu, Thr, Val, Asp, Lys, Ser, Arg, Trp, Gln, Gln, Gly, Asn, Val, Phe, Ser

420 425 430

Cys, Ser, Val, Met, His, Glu, Ala, Leu, His, Asn, His, Tyr, Thr, Gln, Lys, Ser

435 440 445

LeuSerLeuSerProGlyLys

450 455

(210)2

(211)552

(212) PRT

(213) Artificial sequence

(400)2

Met Val Ser Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser

1 5 10 15

Cys, Leu, Leu, Leu, Thr, Gly, Ser, Ser, Ser, Gly, Gly, Arg, Pro, Phe, Val, Glu

20 25 30

Met Tyr Ser Ser Glu Ile Pro Glu Ile Ile His His Met Thr Glu Gly Arg Glu

35 40 45

LeuValIleProCysArgArgValThrThrProAsnIleIleThrThrValThreu

50 55 60

Lys, Lys, Phe, Pro, Leu, Asp, Thr, Leu, Ile, Pro, Asp, Gly, Lys, Arg, Ile, Ile

65 70 75 80

Trp Asp Ser Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu

85 90 95

Ile, Gly, Leu, Leu, Thr, Cys, Glu, Ala, Thr, Val, Asn, Gly, His, Leu, Tyr, Lys

100 105 110

Thr, Asn, Tyr, Leu, Thr, His, Arg, Gln, Thr, Asn, Thr, Ile, Ile, Asp, Val

115 120 125

Leu, Ser, Pro, Ser, His, Gly, Ile, Glu, Leu, Ser, Val, Gly, Glu, Lys, Leu, Val

130 135 140

Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn

145 150 155 160

TrpGluGluTyrProSerSerSysLysHisGlnHisLysLysLysLeuValAsAsArg

165 170 175

Asp, Leu, Lys, Thr, Gln, Ser, Gly, Ser, Glu, Met, Lys, Lys, Phe, Leu, Ser, Thr

180 185 190

Leu Thr Ile Asp Gly Val Thr Arg Ser Ser Asp Gln Gly Leu Tyr Thr Cys

195 200 205

Ala, Ala, Ser, Ser, Gly, Leu, Mer, Thr, Lys, Lys, Asn, Ser, Thr, Phe, Val, Arg

210 215 220

Val, His, Glu, Lys, Pro, Phe, Val, Ala, Phe, Gly, Ser, Gly, Met, Glu, Ser, Leu

225 230 235 240

Val, Glu, Ala, Thr, Val, Gly, Glu, Arg, Val, Arg, Ile, Pro, Ala, Lys, Tyr, Leu

245 250 255

Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Pro Leu

260 265 270

Glu, Ser, Asn, His, Thr, Ile, Lys, Ala, Gly, His, Val, Leu, Thr, Ile, Met, Glu

275 280 285

Val, Ser, Glu, Arg, Asp, Thr, Gly, Asn, Tyr, Thr, Val, Ile, Leu, Thr, Asn, Pro

290 295 300

Ile, Ser, Lys, Glu, Lys, Gln, Ser, His, Val, Val, Ser, Leu, Val, Tyr, Val

305 310 315 320

Pro Pro Gly Pro Pro Gly Asp Lys Thr His His Thr Cys Pro Leu Cys Pro Ala

325 330 335

Pro Glu, Leu, Leu, Gly, Gly, Pro, Ser, Val, Phe, Leu, Phe, Pro, Pro, Lys, Pro

340 345 350

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Val Thr Cys Val Val

355 360 365

Val, Asp, Val, Ser, His, Glu, Asp, Pro, Glu, Val, Lys, Phe, Asn, Trp, Tyr, Val

370 375 380

Asp, Gly, Val, Glu, Val, His, Asn, Ala, Lys, Thr, Lys, Pro, Arg, Glu, Glu, Gln

385 390 395 400

Tyr Asn Ser Thr Tyr Arg Val Val Ser Ser Val Val Leu Thr Val Leu His His Gln

405 410 415

AspTrpLeuAsnGlyLysGluGluTyrLysCysLysValSerAsnLysAla

420 425 430

Leu Pro Ala Pro Ile Glu Lys Thr Thle Ier Ser Lys Ala Lys Gly Gln Pro

435 440 445

Arg, Glu, Pro, Gln, Val, Tyr, Thr, Leu, Pro, Pro, Ser, Arg, Asp, Glu, Leu, Thr

450 455 460

Lys, Asn, Gln, Val, Ser, Leu, Thr, Cys, Leu, Val, Lys, Gly, Phe, Tyr, Pro, Ser

465 470 475 480

Asp, Ile, Ala, Val, Glu, Trp, Glu, Ser, Asn, Gly, Gln, Pro, Glu, Asn, Asn, Tyr

485 490 495

Lys, Ala, Thr, Pro, Val, Leu, Asp, Ser, Asp, Gly, Ser, Phe, Phe, Leu, Tyr

500 505 510

Ser, Lys, Leu, Thr, Val, Asp, Lys, Ser, Arg, Trp, Gln, Gln, Gly, Asn, Val, Phe

515 520 525

Ser, Cys, Ser, Val, Met, His, Glu, Ala, Leu, His, Asn, His, Tyr, Thr, Gln, and Lys

530 535 540

SerLeuSerLeuSerProGlyLys

545 550

(210)3

(211)308

(212) PRT

(213) Artificial sequence

(400)3

GlyArg, Pro, Phe, Val, Glu, Met, Tyr, Ser, Glu, Ile, Pro, Glu, Ile, Ile, His

1 5 10 15

Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Val Thr Ser Pro

20 25 30

Asn, Ile, Thr, Val, Thr, Leu, Lys, Lys, Phe, Pro, Leu, Asp, Thr, Leu, Ile, Pro

35 40 45

Asp Gly Lys Arg Ile I Trp Asp Ser Ser Arg Lys Gly Phe Ile Ile Ser

50 55 60

Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val

65 70 75 80

Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Ash

85 90 95

Thr, Ile, Asp, Val, Val, Leu, Ser, Pro, Ser, His, Gly, Ile, Glu, Leu, Ser

100 105 110

Val Gly, Glu, Lys, Leu, Val, Leu, Asn, Cys, Thr, Ala, Arg, Thr, Glu, Leu, Asn

115 120 125

Val, Gly, Ile, Asp, Phe, Asn, Trp, Glu, Tyr, Pro, Ser, Ser, Lys, His, Gln, His

130 135 140

Lys, Lys, Leu, Val, Asn, Arg, Asp, Leu, Lys, Thr, Gln, Ser, Gly, Ser, Glu, Met

145 150 155 160

Lys, Lys, Phe, Leu, Ser, Thr, Leu, Thr, Ile, Asp, Gly, Val, Thr, Arg, Ser, Asp

165 170 175

Gln, Gly, Leu, Tyr, Thr, Cys, Ala, Ala, Ser, Ser, Gly, Leu, Met, Thr, Lys, Lys

180 185 190

Asn, Ser, Thr, Phe, Val, Arg, Val, His, Glu, Lys, Pro, Phe, Val, Ala, Phe, Gly

195 200 205

Ser, Gly, Met, Glu, Ser, Leu, Val, Glu, Ala, Thr, Val, Gly, Glu, Arg, Val, Arg

210 215 220

IleProAlaLysTyrLeuGlyTyrProProProGluIleLysTrpTyr

225 230 235 240

Lys, Asn, Gly, Ile, Pro, Leu, Glu, Ser, Asn, His, Thr, Ile, Lys, Ala, Gly, His

245 250 255

Val Leu Thr Ile Met Glu Val Ser Ser Glu Arg Asp Thr Gly Asn Tyr Thr

260 265 270

Val, Ile, Leu, Thr, Asn, Pro, Ile, Ser, Lys, Glu, Lys, Gln, Ser, His, Val, Val

275 280 285

Ser Leu Val Val Tyr Val Pro Pro Asp Lys Thr His His Thr Cys Pro Leu

290 295 300

CysProAlaPro

305

(210)4

(211)214

(212) PRT

(213) Artificial sequence

(400)4

GlyArg, Pro, Phe, Val, Glu, Met, Tyr, Ser, Glu, Ile, Pro, Glu, Ile, Ile, His

1 5 10 15

Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Val Thr Ser Pro

20 25 30

Asn, Ile, Thr, Val, Thr, Leu, Lys, Lys, Phe, Pro, Leu, Asp, Thr, Leu, Ile, Pro

35 40 45

Asp Gly Lys Arg Ile I Trp Asp Ser Ser Arg Lys Gly Phe Ile Ile Ser

50 55 60

Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val

65 70 75 80

Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn

85 90 95

Thr, Ile, Asp, Val, Val, Leu, Ser, Pro, Ser, His, Gly, Ile, Glu, Leu, Ser

100 105 110

Val Gly, Glu, Lys, Leu, Val, Leu, Asn, Cys, Thr, Ala, Arg, Thr, Glu, Leu, Asn

115 120 125

Val, Gly, Ile, Asp, Phe, Asn, Trp, Glu, Tyr, Pro, Ser, Ser, Lys, His, Gln, His

130 135 140

Lys, Lys, Leu, Val, Asn, Arg, Asp, Leu, Lys, Thr, Gln, Ser, Gly, Ser, Glu, Met

145 150 155 160

Lys, Lys, Phe, Leu, Ser, Thr, Leu, Thr, Ile, Asp, Gly, Val, Thr, Arg, Ser, Asp

165 170 175

Gln, Gly, Leu, Tyr, Thr, Cys, Ala, Ala, Ser, Ser, Gly, Leu, Met, Thr, Lys, Lys

180 185 190

Asn, Ser, Thr, Phe, Val, Arg, Val, His, Glu, Lys, Asp, Lys, Thr, His, Thr, Cys

195 200 205

Pro LeuCys Pro Ala Pro

210

CN102233132B Application of VEGF acceptor fusion proteins in preparation of drugs for inhibiting growth of ocular surface neovascularization

1.The VEGFR fusion protein FP3 is used in the preparation of a medicine for treating conjunctivitis. The amino acid sequence of FP3 is shown in SEQ ID NO: 2.

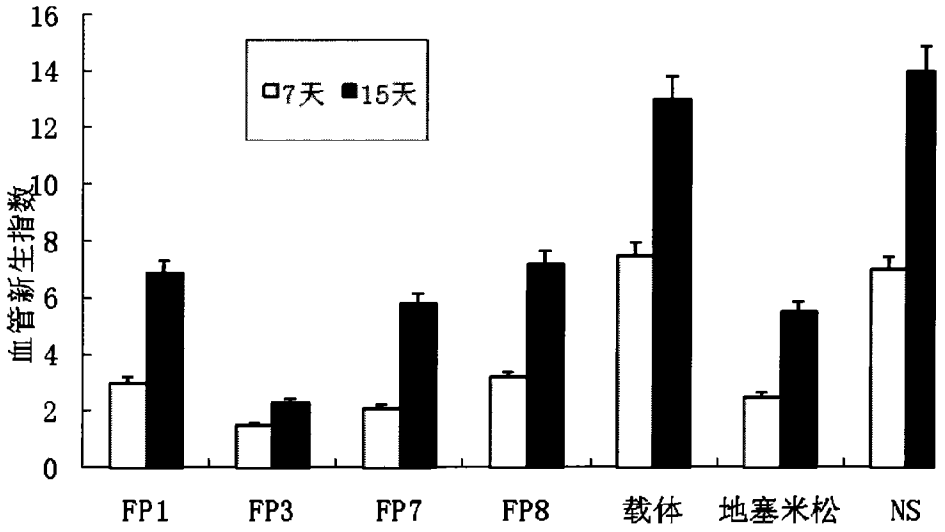


图 1

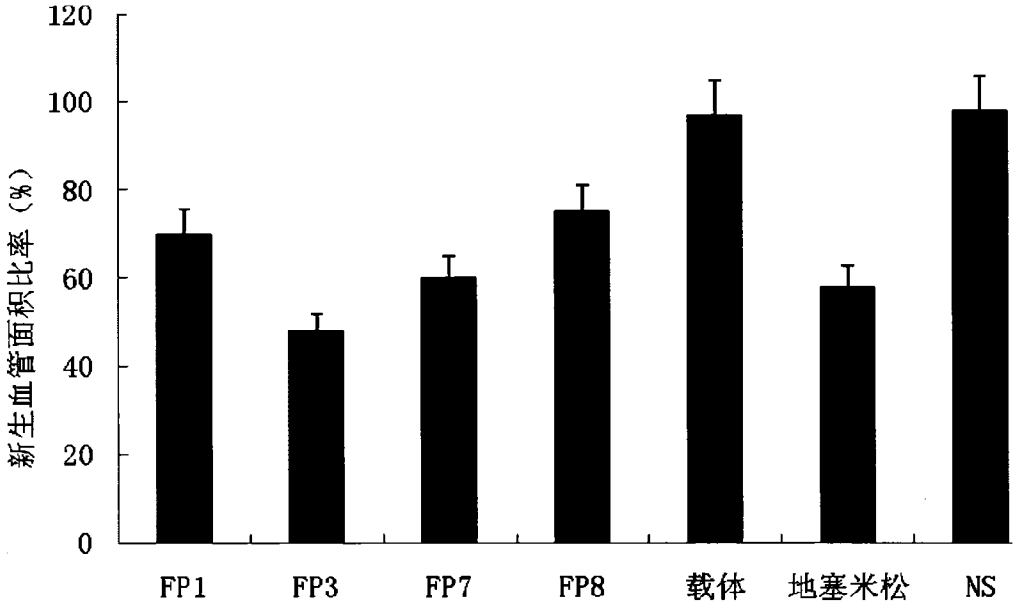


图 2

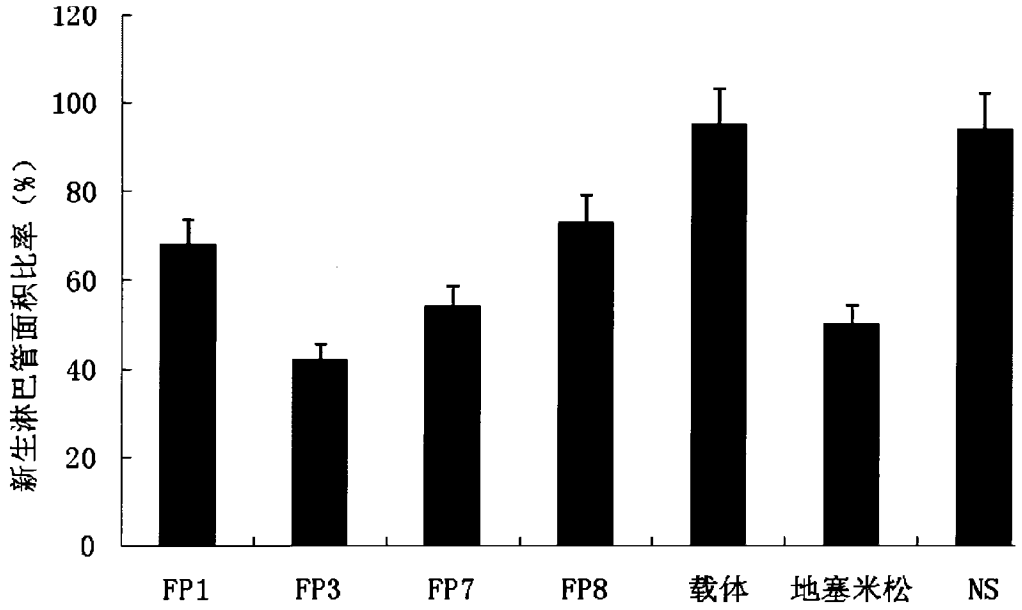


图 3

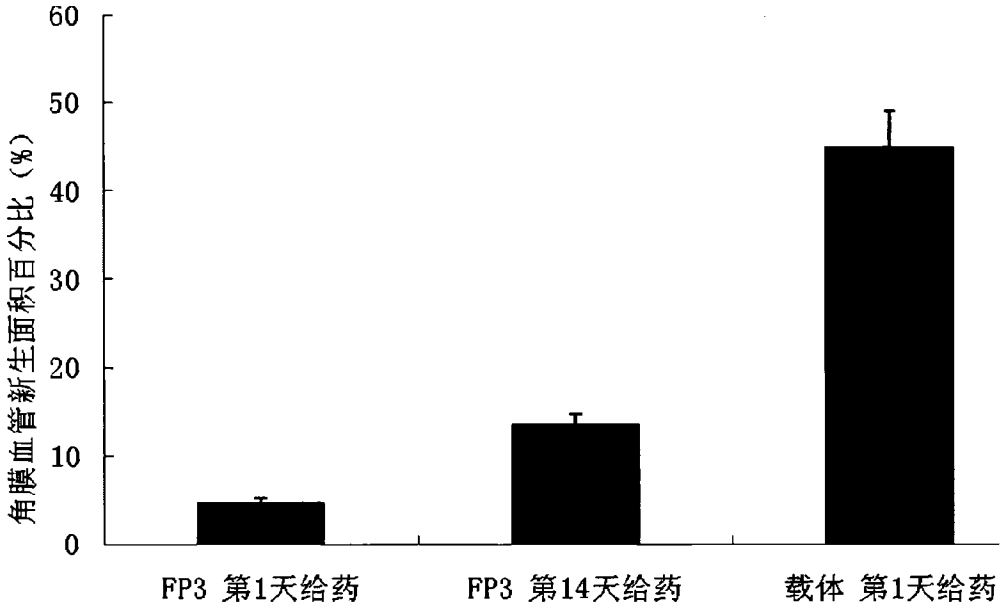


图 4

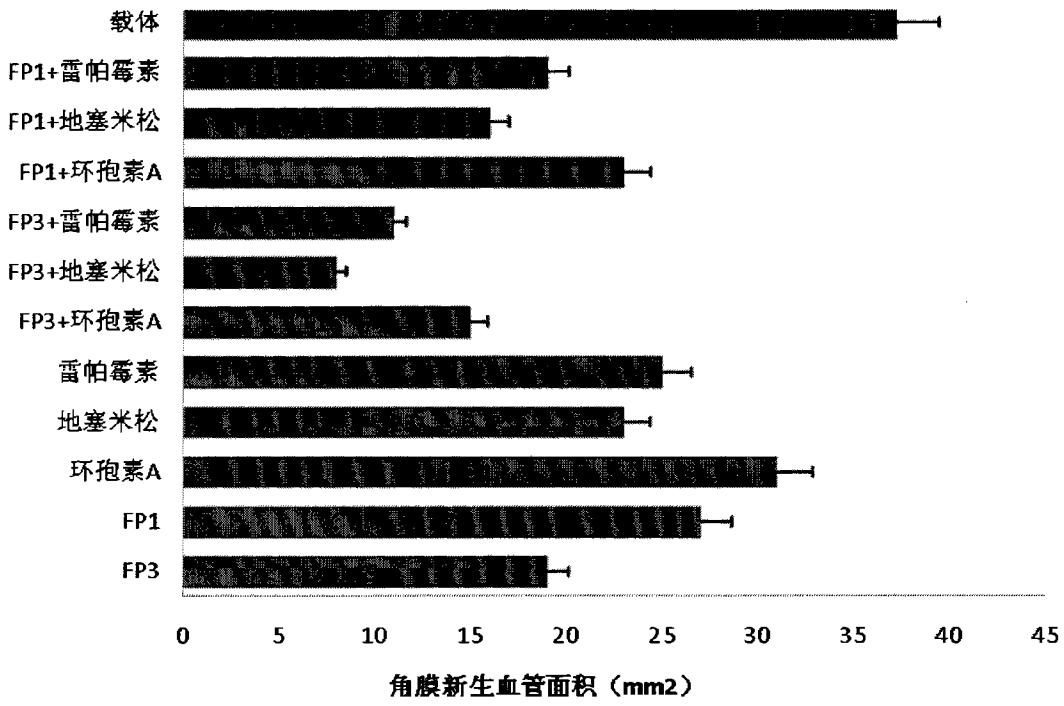


图 5

(19)中华人民共和国国家知识产权局

(12)发明专利

(10)授权公告号 CN 102233132 B
(45)授权公告日 2013.10.23

(21)申请号 201010158098.5

(22)申请日 2010.04.28

(73)专利权人 成都康弘生物科技有限公司
地址 610036 四川省成都市金牛区蜀西路36号

(72)发明人 俞德超

(51)Int.Cl.
A61K 39/395(2006.01)
A61K 45/00(2006.01)
A61P 27/02(2006.01)
A61P 27/06(2006.01)
A61P 31/04(2006.01)
A61P 31/12(2006.01)
A61P 31/22(2006.01)
A61K 38/18(2006.01)
A61K 31/573(2006.01)

(56)对比文件
CN 1915427 A,2007.02.21,
Balamurali K.Ambati et al..Corneal avascularity is due to soluble VEGF receptor-1.《Nature》.2006,第443卷(第26期),993-997.
林志荣等.上皮缺损诱导角膜新生血管小鼠模型中VEGF与sFlt_1变化趋势的实验研究.《中国眼耳鼻喉科杂志》.2009,第9卷(第2期),80-84.

审查员 吕小蒙

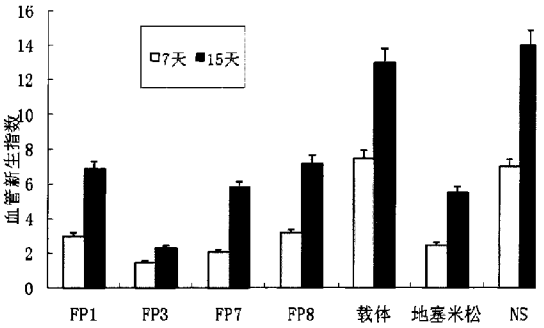
权利要求书1页 说明书10页
序列表6页 附图3页

(54)发明名称

VEGF受体融合蛋白在制备抑制眼表新生血管生长的药物中的应用

(57)摘要

本发明涉及VEGFR融合蛋白FP1、FP3、FP7、FP8用于制备治疗眼表新生血管相关病症的药物中的应用,以及融合蛋白FP1、FP3、FP7和FP8与免疫抑制剂的联合制剂,所述的免疫抑制剂选自皮质类固醇、雷帕霉素、地塞米松、环孢素A的任一种或其组合,其中,FP1、FP3、FP7和FP8的氨基酸序列分别如SEQ ID NO:1、2、3、4所示。



CN 102233132 B

1. VEGFR融合蛋白FP3用于制备治疗结膜炎的药物中的应用，其中FP3的氨基酸序列如SEQ ID NO：2所示。

VEGF 受体融合蛋白在制备抑制眼表新生血管生长的药物中的应用

技术领域

[0001] 本发明涉及血管内皮细胞受体(Vascular Endothelial Growth Factor receptor，VEGFR)融合蛋白的医药用途，特别涉及 VEGFR 融合蛋白用于制备治疗由眼表新生血管生长引起的疾病的药物中的应用。

背景技术

[0002] 眼表的角膜和结膜很容易受到各种刺激，包括微生物感染、物理撞击、化学成分损伤、角膜接触镜引起角膜摩擦破损、角膜缺氧等，都会导致角膜或者结膜发生病变，这些疾病往往产生眼表新生血管。与眼表新生血管相关的疾病包括角膜新生血管、新生血管性青光眼、翼状胬肉、结膜炎、细菌角膜炎、真菌性角膜炎、病毒性角膜炎、泡性角膜结膜炎、角膜溃疡、巩膜炎、角膜接触镜并发症等。

[0003] 角膜新生血管疾病包括角膜外伤（包括眼表的刺透伤、破裂伤、划伤、昆虫叮咬伤、化学烧伤、物理烧伤等）、角膜移植排斥、眼前段及后段手术引起的眼表损伤、角膜基质溃疡瘢痕形成、异物刺激、隐形眼镜有关的缺氧导致角膜新生血管，角膜缘干细胞缺乏、营养不良和维生素缺乏症、药物不良反应及医源性损伤等原因引起的血管新生疾病。

[0004] 角膜是眼球重要的屈光间质之一，在正常状态下是透明和无血管的，但是，角膜在感染、损伤、缺氧和角膜移植等病理状态下，毛细血管从角膜缘向角膜内生长，形成角膜的新生血管。尽管角膜新生血管的发生有利于局部清除感染和促进损伤修复(Curr Opin Ophthalmol.12:242-249,2001)，但角膜新生血管的发生可引起角膜组织的免疫特性环境的改变(Exp EyeRes.78:579-589,2004)，最终引发病人视力下降、角膜损害和眼表屏蔽功能丧失等临床问题。角膜新生血管常导致持续性炎症和组织的瘢痕化，角膜新生血管是许多致盲性角膜病病变中的主要特征，也是角膜感染、化学损伤和角膜移植术后的主要视力损害的并发症。同时，新生血管也影响角膜移植的成功率。

[0005] 目前，发达国家角膜新生血管引起的眼部疾病占失明原因的首位，其失盲率达到57.2%。我国由眼外伤所致的角膜新生血管约占整个角膜疾病的10%，因此，角膜新生血管的预防和治疗成为目前急需解决的问题。

[0006] 尽管激光治疗和外科干预为角膜新生血管病人提供潜在的治疗方法，但治疗后仍留下一些难题。例如，多数情况下，经过激光治疗和外科干预的病人的角膜的无血管状态和透明状态不能重建。此外，临床目前用于治疗角膜新生血管的药物主要是地塞米松等激素药物，该类激素药物存在用药后扩大角膜损伤面积，角膜愈合速度慢，副反应较多等缺陷。因此，提供一个安全有效的治疗药物，用于预防和早期治疗干预眼表新生血管疾病和眼表炎症具有十分重要的意义。

[0007] 角膜新生血管的发生是一个复杂的病理过程，已有研究结果证实，角膜新生血管发生和发展的主要原因是由于眼表组织中血管生成因子和抗血管生成因子之间的平衡发生倾斜，一些眼表疾病可继发性高表达促血管生长的细胞因子，诱导眼表向有利于角膜新

生血管形成的方向发展，其中，血管内皮生长因子为目前发现的最重要的促血管内皮细胞生长因子 (Science246 :1306-9，1989)。

[0008] 中国专利 ZL200510073595.4 公开了融合蛋白 FP1-FP6 结构和制备方法及其抗肿瘤用途，以及用于视网膜病变，但未涉及角膜新生血管的防治。中国专利 ZL200610066257.2 公开了融合蛋白 FP1-FP8 的结构及其制备方法，以及其在制备治疗由新生血管生长而引发的眼科疾病中的药物中的应用，同样没有涉及 VEGFR 融合蛋白 FP1、FP3、FP7 和 FP8 在制备治疗角膜新生血管的药物中应用。

[0009] 新生血管性青光眼是指虹膜和小梁表面有新生的纤维血管膜所导致周边虹膜前粘连，阻碍房水排出引起的青光眼，其新生血管容易破裂，反复发生前房出血，故又称出血性青光眼。该类疾病是一种病情重、易复发、较为顽固的继发性青光眼，目前一般的抗青光眼药物及手术治疗往往难于凑效，尽管多采用激光或冷冻手术治疗，但易复发，甚至导致失明。患者眼部充血，角膜水肿，剧烈眼痛、头痛，为了解除痛苦，部分患者不得不摘除眼球。

[0010] 翼状胬肉的病因至今未明，可能与紫外线照射、烟尘污染有关。近年来，有研究认为，该病与新生血管有关，和过敏反应以及弹力纤维变性也有一定关系。目前，对该类疾病没有较好的治疗方法，尽管可以手术治疗，但对患者的影响较大，急需安全有效的防治药物。

[0011] 结膜炎是眼科常见病、多发病。其中，对于急性结膜炎的防治主要使用抗生素或激素。慢性结膜炎易复发，难以治愈，导致患者眼睛干涩、异物感，长期患病会导致视力下降、血管增生，也会累及角膜，引起角膜炎，角膜溃疡等。该类疾病的有效防治也急需安全有效的防治药物。

[0012] 中国专利 ZL200510073595.4 和 ZL200610066257.2 公开的技术内容均作为本申请的参考。

发明内容

[0013] 本发明的一个目的在于提供一种 VEGFR 融合蛋白 FP1、FP3、FP7、FP8 用于制备治疗眼表新生血管相关病症的药物中的应用，其中，FP1、FP3、FP7 和 FP8 的氨基酸序列分别如 SEQ ID NO :1、2、3、4 所示。

[0014] 在本发明的一个优选实施方案中，所述的眼表新生血管相关病症选自角膜新生血管、结膜新生血管、结膜炎、翼状胬肉、新生血管性青光眼、角膜炎、细菌角膜炎、真菌性角膜炎、病毒性角膜炎、角膜溃疡、泡性角膜结膜炎、巩膜炎、角膜接触镜并发症的任一种或其并发症，优选为角膜新生血管、结膜新生血管的任一种或其并发症。

[0015] 在本发明的一个实施方案中，所述的角膜新生血管相关病症选自角膜外伤、角膜移植排斥、眼前段及后段手术引起的眼表损伤、角膜基质溃疡瘢痕形成、异物刺激、缺氧导致角膜新生血管增生，以及角膜缘干细胞缺乏、营养不良、维生素缺乏、药物不良反应或医源性损伤所致的新生血管疾病的任一种或其并发症。

[0016] 在本发明的优选实施方案中，所述的角膜外伤选自眼表的刺透伤、破裂伤、划伤、昆虫叮咬伤、化学烧伤、物理烧伤的任一种或其并发症。

[0017] 在本发明的优选实施方案中，所述的化学烧伤选自酸烧伤或碱烧伤。

[0018] 在本发明的优选实施方案中，所述的病毒性角膜炎选自单纯疱疹病毒 (HSV) 感染

引起的病毒性角膜炎。

[0019] 在本发明的优选实施方案中，所述的角膜新生血管相关病症选自单纯疱疹病毒感染、手术缝合线、角膜移植术后、酸烧伤或碱烧伤的任一种诱发或引起的角膜新生血管生长。

[0020] 本发明的VEGFR融合蛋白FP1、FP3、FP7和FP8通过捕获VEGF家族的多个亚类（如VEGF-A，VEGF-B，VEGF-C，VEGF-D）和胎盘生长因子（PIGF），封闭和抑制其受体的活化作用，从而表现封闭或抑制VEGF蛋白生物活性的作用。

[0021] 本发明所述的VEGFR融合蛋白FP1、FP3、FP7和FP8均由人不同VEGFR功能区蛋白和/或人IgG FC片段蛋白融合而成。具体而言，FP1由VEGFR1的第2免疫球蛋白样区与VEGFR2的第3免疫球蛋白样区组成；FP3由VEGFR1的第2免疫球蛋白样区、VEGFR2的第3和第4免疫球蛋白样区、以及人IgG免疫球蛋白的FC段蛋白组成；FP7由VEGFR1的第2免疫球蛋白样区、VEGFR2的第3和第4免疫球蛋白样区、以及人IgG免疫球蛋白的FC段蛋白组成；FP8由VEGFR1的第2免疫球蛋白样区、VEGFR2的第3免疫球蛋白样区、以及人IgG免疫球蛋白的FC段蛋白组成。

[0022] 本发明提供的方法也可用于治疗角膜急性期损伤和亚急性期损伤。急性期损伤为伤后1周内，临床表现为眼表组织的水肿和血栓形成，以及受累的角膜、结膜和血管内皮细胞出现变性和坏死。亚急性期一般为伤后2周至6个月内，临床表现为眼表各种细胞增生和迁移，修补损伤和缺损，并出现角膜上皮细胞被结膜上皮所取代，以及角膜有大量新生血管（NV）形成，损伤组织下有大量白细胞侵入。在各种具体应用方面，急性期损伤的治疗应在损伤后24小时内进行，亚急性期损伤的治疗应根据眼表局部综合情况，选择最佳治疗时间。

[0023] 本发明的另一目的在于提供VEGFR融合蛋白FP1、FP3、FP7和FP8与免疫抑制剂的联合制剂，所述的免疫抑制剂选自皮质类固醇、雷帕霉素、地塞米松、环孢素A的任一种或其组合，优选为地塞米松。

[0024] 本发明所述的联合制剂可以顺序给药或先后给药，也就是说，医务人员在为患者给药时，可以先给患者使用VEGFR融合蛋白FP1、FP3、FP7和FP8制剂，再给患者使用本发明所述的免疫抑制剂；或者先给患者使用本发明所述的免疫抑制剂，再给患者使用VEGFR融合蛋白FP1、FP3、FP7和FP8制剂；或者同时给患者使用VEGFR融合蛋白FP1、FP3、FP7和FP8制剂和本发明所述的免疫抑制剂。

附图说明

[0025] 图1融合蛋白FP1、FP3、FP7和FP8对碱烧伤诱发的大鼠角膜新生血管的影响

[0026] 图2融合蛋白FP1、FP3、FP7和FP8对手术缝合线诱发的小鼠角膜血管新生的抑制作用

[0027] 图3融合蛋白FP1、FP3、FP7和FP8对手术缝合线诱发的小鼠角膜淋巴管新生的抑制作用

[0028] 图4融合蛋白FP3对碱烧伤诱发的兔角膜血管新生的抑制作用

[0029] 图5融合蛋白FP1、FP3和免疫抑制剂对大鼠角膜移植术后角膜血管新生的抑制作用

具体实施方式

[0030] 以下通过实施例进一步说明本发明，但不应理解为构成对本发明的任何限制。

[0031] 实施例 1 融合蛋白对碱烧伤诱发的角膜血管新生的治疗作用及安全性试验

[0032] 采用碱烧伤方法建立大鼠 (SD 大鼠，雌性，体重为 250g±20g) 角膜血管新生的动物模型，研究融合蛋白 FP1、FP3、FP7 和 FP8 对碱烧伤诱发的大鼠角膜血管新生的抑制作用。

[0033] 取大白鼠，在麻醉状态下，以右眼为实验眼，用直径为 3mm 浸透了 1mol/L NaOH 溶液的滤纸片贴于大鼠的右眼角膜中央，作用 1 分钟后去除滤纸片，用大量生理盐水冲洗结膜囊，去除残余的 NaOH，并滴加氯霉素滴眼液 (生产厂家 : 常州四药制药有限公司，规格 8ml ： 20mg)20ul 和 1％阿托品眼膏。

[0034] 次日，将大鼠平均分为 7 组 (每组 6 只)，即 FP1 组、FP3 组、FP7 组、FP8 组、药物载体组 (即 PBS 溶液)、地塞米松组和生理盐水组 (NS)。其中，FP1、FP3、FP7 和 FP8 组中融合蛋白的给药浓度均为 20mg/ml，临用前用 PBS 溶液配置，其中，PBS 由 0.24g 磷酸二氢钾 (KH2PO4)，1.44g 磷酸氢二钠 (Na_2HPO_4)，8.0g 氯化钠 (NaCl)，0.2g 氯化钾 (KCl)，加水至 1000mL，加 NaOH 调节 pH7.4 ；地塞米松组的给药浓度为 0.25mg/ml。每口在各组动物眼表滴加各组溶液 4 次，每次 50 微升，给药时间分别为 8:00、12:00、16:00、20:00，连续应用 14 天。同时，在造模后 15 天进行角膜荧光染色照相，观察药物对角膜碱烧伤后形成的溃疡面积的影响，评价药物的安全性。

[0035] 在碱烧伤后第 15 天，将动物麻醉，用墨水染色角膜新生血管，在显微镜下观察并采集图像，并按 Robert 公式 $A = C/12 \times 3.1416[R^2-(R-L)^2]$ 计算角膜新生血管生长面积，其中，A 代表新生血管面积，C 为新生血管累积角膜的圆周钟点数，L 为新生血管从角膜缘伸入角膜的长度，R 为受试动物角膜平均半径 (本次实验为 2.6mm)，结果见表 1。

[0036] 表 1 融合蛋白对角膜碱烧伤后角膜新生血管的抑制作用

[0037]

组别	角膜新生血管面积 (mm^2)
FP1 治疗组	7.4±1.5
FP3 治疗组	3.0±0.7
FP7 治疗组	4.2±1.1
FP8 治疗组	4.1±1.3
地塞米松组	9.8±1.0
药物载体组	10.2±1.6
生理盐水组	11.0±1.2

[0038] 表 1 结果显示 : 与地塞米松组相比，VEGFR 融合蛋白 FP1、FP3、FP7 和 FP8 能够明显抑制角膜血管的新生，可用于治疗眼表烧伤性疾病，并且，融合蛋白 FP3 对眼表血管新生的

抑制能力相对更强，对大鼠角膜新生血管长度和面积的抑制作用更为显著。并且融合蛋白治疗组动物的角膜水肿程度较轻，新生血管较细小并分布稀疏，多位于角膜缘附近，血管未进入角膜中央烧伤区，并且，FP3 组的大鼠角膜新生血管长度和面积比均明显小于其他治疗组、药物载体组和生理盐水组 ($p < 0.05$)，也显著优于地塞米松组。

[0039] 表 2 融合蛋白对角膜碱烧伤后角膜损伤的影响

[0040]

组别	用药 14 天后角膜损伤面积 (mm^2)
FP1 治疗组	2.5±0.4
FP3 治疗组	2.0±0.9
FP7 治疗组	2.4±1.3
FP8 治疗组	2.7±1.2
地塞米松组	9.4±1.4
药物载体组	2.3±1.2
生理盐水组	2.1±1.1

[0041] 地塞米松治疗组在连续用药 14 天后，虽然角膜新生血管得到了抑制，但角膜损伤加重，损伤面积溃疡。而融合蛋白治疗组的角膜损伤没有加重的趋势，与生理盐水对照组相当，具有很好的安全性。

[0042] 由此可见，融合蛋白组抑制了新生血管同时没有地塞米松存在的抑制角膜愈合的副作用，疗效和安全性都优于地塞米松。

[0043] 实施例 2 融合蛋白对单纯疱疹病毒角膜炎的治疗作用和安全性考察

[0044] 采用角膜划痕法，制备小鼠 (BalB/C 小鼠，雌性，6-8 周龄，体重 20-25g) 角膜损伤感染单纯疱疹病毒的动物模型。

[0045] 单纯疱疹病毒在 vero 细胞上传代培养，通过常规方法定量。小鼠右眼为实验眼，在麻醉状态下，用蘸有 2μl HSV-1 型病毒液体（约含 5×10^5PFU 单纯疱疹 1 型病毒）的微量加样针，在动物角膜上轻轻划痕并按摩结膜。

[0046] 将实验动物分为 7 组（每组 8 只），即 FP1 组、FP3 组、FP7 组、FP8 组、药物载体组、地塞米松组和生理盐水组 (NS)。其中，FP1、FP3、FP7 和 FP8 组中融合蛋白的给药浓度均为 10mg/ml，临用前用 PBS 溶液稀释配置；药物载体组为 PBS 溶液；地塞米松组的给药浓度为 0.25mg/ml。每日在各组动物眼表滴加各组溶液 4 次，每次 10 微升，给药时间分别为 8:00、12:00、16:00、20:00，连续给药 14 天。

[0047] 在治疗结束后，将动物麻醉，用裂隙灯观察眼表血管新生和损伤程度。各组动物的角膜损伤用损伤指数评价，具体损伤指数按以下标准划分，0：正常角膜；1：轻微混浊；2：中度混浊；3：重度混浊（虹膜看不清）；4：重度混浊伴溃疡；5：角膜破裂。每组动物在每个损伤等级的动物数量的分布情况如表 3 所示。其中，损伤指数小表示对眼表损伤越小，损伤指

数小的动物数量越少表明药物对角膜的损伤越小和保护越好。

[0048] 结果显示，用药结束后，FP1 组、FP3 组、FP7 组、FP8 组损伤指数≤ 2 的大鼠分别为 50％、75％、62.5％和 50％，药物载体组和生理盐水组均为 25％，地塞米松组为 25％，表明 FP3 治疗病毒性角膜炎效果较好且对于角膜损伤没有扩大，有较好的保护作用。

[0049] 表 3 角膜损伤指数的动物统计

[0050]

组别	损伤指数≤ 1	损伤指数＝ 2	损伤指数＝ 3	损伤指数＝ 4
FP1 治疗组	25％	25％	25％	25％
FP3 治疗组	37.5％	37.5％	12.5％	12.5％
FP7 治疗组	25％	37.5％	25％	12.5％
FP8 治疗组	25％	25％	12.5％	37.5％
地塞米松组	12.5％	12.5％	50％	25％
药物载体组	12.5％	12.5％	25％	50％
生理盐水组	12.5％	12.5％	25％	50％

[0051] 角膜血管新生程度用血管新生指数评价，具体血管新生指数划分主要基于新生血管侵犯角膜的范围，采用 16 分制，即将角膜分成 16 区（角膜的 4 个象限和每个象限内的 4 个区），血管侵犯 1 个区得 1 分，最终血管新生指数为血管侵犯角膜各区数量的总和。

[0052] 在给药的第 7 天和第 15 天分别进行观察，计算各组动物角膜新生血管的血管新生指数。与药物载体组和生理盐水组相比，FP1 组、FP3 组、FP7 组、FP8 组动物角膜血管新生指数的增长趋势明显降低 ($p < 0.05$)，并且，FP3 蛋白的作用能力最强且明显优于地塞米松组，结果见图 1。

[0053] 可见，融合蛋白 FP1、FP3、FP7、FP8 能明显抑制角膜血管新生和降低角膜损伤程度，可用于治疗病毒性角膜炎，并且安全性。

[0054] 实施例 3 融合蛋白对手术缝合线诱发的小鼠角膜血管新生的抑制作用

[0055] 采用小鼠角膜基质内种植手术缝合线法建立角膜血管新生的动物模型。

[0056] 取 6 周龄雄性 BalB/c 小鼠，体重 18-25g，在麻醉状态下，以右眼为实验眼，用将 3 根手术缝线（11-0 尼龙线）种植在角膜基质中（每根线起于角膜缘附近，止于角膜中央区，相邻两线夹角为 120° ）。

[0057] 在种植缝线 3 天后，将动物分成以下 7 组（每组 6 只），即 FP1 组、FP3 组、FP7 组、FP8 组、药物载体组、地塞米松组和生理盐水组 (NS)。其中，FP1、FP3、FP7 和 FP8 组中融合蛋白的给药浓度均为 10mg/ml，临用前用 PBS 溶液稀释配置；药物载体组为 PBS 溶液；地塞米松组的给药浓度为 0.25mg/ml。每日在各组动物眼表滴加各组溶液 4 次，每次 50 微升，给药时间分别为 8:00、12:00、16:00、20:00，连续应用 14 天。

[0058] 治疗结束后，处死动物，取角膜组织进行免疫组化染色。用 FITC（异硫氰酸荧光素

fluorescein isothiocyanate)标记CD31(血小板内皮细胞粘附分子-1)显示新生血管,用Cy3(吲哚菁绿染料)标记LYVE-1(淋巴管内皮细胞透明质酸受体-1)显示新生淋巴管,在荧光显微镜下,将角膜分成8个区,依次采集每个区的荧光图象(100×),用图像分析软件计算新生血管和淋巴管的面积,以生理盐水组小鼠角膜新生血管或淋巴管面积为100%,分析各组动物角膜新生血管或淋巴管生长情况,并以新生血管或淋巴管面积比率表示。

[0059] 结果显示:通过眼表应用含有融合蛋白FP3的滴眼液,能明显减少缝合手术线诱导的角膜血管和淋巴管新生。并且,FP3组的小鼠角膜新生血管($p < 0.05$)和新生淋巴管面积比率($p < 0.05$)均明显小于对照组,见图2和图3。

[0060] 可见,融合蛋白FP1、FP3、FP7、FP8能明显抑制角膜血管和淋巴管的新生,可用于治疗外伤性眼表新生血管性疾病、眼表炎症、角膜接触镜引起的角膜炎等。

[0061] 实施例4 结膜下注射融合蛋白FP3对碱烧伤诱发兔角膜血管新生的抑制作用

[0062] 同实施例2,采用碱烧伤方法建立兔角膜血管新生的动物模型。

[0063] 取新西兰大白兔18只,雌雄不限,体重2.0-2.5kg。在麻醉状态下,以右眼为实验眼,用浸透NaOH(浓度为1mol/L)溶液的滤纸片(直径为5mm)贴于兔的右眼角膜中央,作用1分钟后去除滤纸片,用10ml生理盐水冲洗结膜囊,去除残余的NaOH,并滴加氯霉素滴眼液(生产厂家:常州四药制药有限公司,规格8ml : 20mg)20ul。

[0064] 将造模动物随机分为以下3组(每组6只),即FP3第一天给药组,碱烧伤后第1天结膜下注射FP3;FP3第14天给药组,碱烧伤后第14天结膜下注射FP3;药物载体PBS溶液对照组,碱烧伤后第1天注射药物载体。FP3治疗组的给药体积为0.05ml,给药浓度为10mg/ml,对照组的给药体积同治疗组。

[0065] 21天后,将动物麻醉,用显微镜观察兔眼角膜,并测量角膜部位新生血管的面积,用血管新生面积占整个眼表的面积百分率表示血管新生程度,结果见图4。

[0066] 可见,FP3第一天给药组动物的角膜新生血管面积百分比明显小于对照组($p < 0.01$)和FP3第14天给药组($p < 0.05$),说明FP3能明显抑制碱烧伤所致的角膜血管新生,且早期使用对损伤血管新生的抑制效果更好。

[0067] 实施例5 融合蛋白对酸烧伤引起的大鼠角膜新生血管的抑制作用

[0068] 采用酸烧伤法建立大鼠(SD大鼠,雌性,体重200-250g)角膜新生血管模型。

[0069] 用同一支含有脱脂棉芯的内径为1.5mm的加样枪头,蘸取750g/L硝酸银和250g/L硝酸钾混合烧灼液,将其放在大鼠角膜(随机烧灼右眼或左眼角膜)中央10s,移开加样枪头,立即用生理盐水冲洗1min。

[0070] 次日,将造模大鼠(体重为180g±20g)随机分为以下6组(每组6只),即FP1组、FP3组、FP7组、药物载体组、地塞米松组和生理盐水组(NS)。其中,FP1、FP3、FP7组中融合蛋白的给药浓度均为10mg/ml,临用前用PBS溶液稀释配置;药物载体组为PBS溶液;地塞米松组的给药浓度为0.25mg/ml。每日在各组动物眼表滴加各组溶液4次,每次20微升,给药时间分别为8:00、12:00、16:00、20:00,连续给药14天。

[0071] 术后15天,用氯胺酮(60mg/kg)和氯丙嗪(30mg/kg)腹腔内注射麻醉后,于裂隙灯显微镜下采集图像,测量角膜新生血管长度和钟点数。

[0072] 角膜新生血管生长面积按Robert公式$A = C/12 \times 3.1416[R^2-(R-L)^2]$计算,其中A代表新生血管面积,C为新生血管累积角膜的圆周钟点数,L为新生血管从角膜缘伸入角

膜的长度，R 为受试动物角膜平均半径（本次实验为 3mm）。

[0073] 结果显示：术后 15 天，融合蛋白治疗组动物的角膜水肿程度较轻，新生血管较细小并分布稀疏，多位于上、下角膜缘附近。并且，与对照组相比，FP1 组、FP3 组、FP7 组大鼠的角膜新生血管长度和面积显著降低（$p < 0.05$），结果见表 4。

[0074] 可见，融合蛋白 FP1、FP3、FP7 对酸烧伤引起的炎症性角膜新生血管有明显抑制作用，可用于治疗眼表炎性疾病，且 FP3 对眼表炎性病症血管新生的抑制效果更佳。

[0075] 表 4 融合蛋白对角膜酸烧伤后角膜新生血管的抑制作用

[0076]

组别	角膜新生血管面积（mm^2）
FP1 治疗组	6.7±1.5
FP3 治疗组	3.0±0.5
FP7 治疗组	4.3±0.6
地塞米松组	8.8±1.1
药物载体组	10.9±1.5
生理盐水组	11.2±1.6.

[0077] 实施例 6 融合蛋白对结膜炎的治疗作用

[0078] 采用乙醇诱导法制备家兔结膜炎动物模型。

[0079] 选取日本大耳白家兔 42 只，雌雄不限，体重 1.8-2.2kg。随机分成 7 组，每组 6 只，即 FP1 组、FP3 组、FP7 组、FP8 组、药物载体 PBS 溶液组、地塞米松组和生理盐水组（NS）。

[0080] 将家兔左眼下睑拉成杯状，用微量加样器滴入医用级无水乙醇于眼结膜囊内，第 1 天滴加 0.135ml，第 2 天滴加 0.09ml，第 3 天滴加 0.045ml，右眼作为自身对照。第 4 天进行观察，出现结膜充血、水肿、闭目微光、分泌物增加、睫状充血则视为造模成功。

[0081] 造模成功后给予各组药物，其中，FP1、FP3、FP7 和 FP8 组中融合蛋白的给药浓度均为 10mg/ml，临用前用 PBS 溶液稀释配置；药物载体组为 PBS 溶液；地塞米松组的给药浓度为 0.25mg/ml。每日在各组动物眼表滴加各组溶液滴加各组溶液 4 次，每次 50 微升，给药时间分别为 8:00、12:00、16:00、20:00，连续应用 7 天。

[0082] 在治疗结束后，观察动物结膜及眼表情况。各组动物的结膜炎严重程度按以下标准划分：1. 结膜充血：无充血（-）；轻度充血（+）；明显充血，呈深红色（++）；弥漫性充血，呈紫红色（+++）；2. 眼睑水肿：无水肿（-）；轻度水肿（+）；明显充血，部分眼睑外翻（++）；明显水肿，眼睑半闭（+++）；3. 分泌物：无异常分泌物（-）；少量分泌物（+）；分泌物使眼睑或睫毛潮湿或粘着（++）；分泌物使整个眼区潮湿或粘着（+++），结果见表 5。

[0083] 表 5 融合蛋白对家兔结膜炎的影响

[0084]

组别	造模后结膜情况			治疗7天后结膜情况		
	充血	水肿	分泌物	充血	水肿	分泌物
FP1	+++	+++	+++	+	++	+
FP3	+++	+++	+++	-	-	-
FP7	+++	+++	+++	-	+	+
FP8	+++	+++	+++	++	+	+
地塞米松	+++	+++	+++	+	+	-
药物载体	+++	+++	+++	+++	++	+++
生理盐水	+++	+++	+++	+++	+++	++

[0085] 结果显示，用药结束后，融合蛋白治疗组和地塞米松组均能显著改善结膜炎症状，其中 FP3 对结膜炎的改善效果最好。

[0086] 实施例 7 融合蛋白对大鼠角膜移植术后角膜血管新生的抑制作用

[0087] 通过制作大鼠角膜移植模型研究融合蛋白在角膜移植术后血管新生中的抑制作用。

[0088] 用 48 只 Wistar 雌性大鼠（体重为 180g±20g）为供体，96 只 SD 雌性大鼠（体重为 180g±20g）为受体，鼠龄 2-3 个月。受体动物右眼接受角膜移植，供体双眼取材后处死。供体提供双眼角膜，受体均选择右眼手术。供体、受体术前 20 分钟用 1％阿托品滴眼液和 0.5％托品酰胺滴眼液散瞳，10％水合氯醛以 3ml/kg 腹腔注射麻醉，同时眼局部用表面麻醉剂麻醉。于供体角膜钻取直径为 3.5mm 植片，置于培养皿中并用粘弹剂保护植片备用。于受体右眼，用直径为 3.0mm 的环钻钻受体植床，植床植片间用无损伤缝线作间断缝合 8 针。线结暴露不包埋。然后，前房注射平衡液以形成前房。术毕散瞳，结膜下注射庆大霉素 0.1ml，结膜囊内涂红霉素眼膏。丝线缝合睑缘 1 针，并于术后第 1 天拆除。

[0089] 次日，将造模受体大鼠随机分为 12 组（每组 8 只），即 FP3 组、FP1 组、地塞米松组、环孢素 A 组、雷帕霉素组、地塞米松加 FP3 组、环孢素 A 加 FP3 组、雷帕霉素加 FP3 组、地塞米松加 FP1 组、环孢素 A 加 FP1 组、雷帕霉素加 FP1 组、药物载体 PBS 溶液组。其中，FP1、FP3 组中融合蛋白的给药浓度均为 10mg/ml，临用前用 PBS 溶液稀释配置；药物载体组为 PBS 溶液；地塞米松组的给药浓度为 0.25mg/ml；环孢素 A 的给药浓度为 1mg/ml；雷帕霉素的给药浓度 1mg/ml，均为眼表给药。其中，FP3 组、FP1 组、地塞米松组、环孢素 A 组、雷帕霉素组、药物载体组每日给药 4 次，每次 20ul，给药时间分别为 8:00、12:00、16:00、20:00，连续应用 14 天；地塞米松加 FP3 组、环孢素 A 加 FP3 组、雷帕霉素加 FP3 组、地塞米松加 FP1 组、环孢素 A 加 FP1 组、雷帕霉素加 FP1 组采取顺序给药或者先后给药方式，FP1 和 FP3 蛋白给药为每天早晚各 1 次，每次 20ul，化学药物每天给药一次，每次 20ul，连续应用 14 天。

[0090] 术后 15 天用氯胺酮 (60mg/kg) 和氯丙嗪 (30mg/kg) 腹腔内注射麻醉后，于裂隙灯显微镜下采集图像，测量角膜新生血管长度和钟点数。

[0091] 角膜新生血管生长面积按 Robert 公式 $A = C/12\times3.1416[R^2-(R-L)^2]$ 计算，其中 A 代表新生血管面积，C 为新生血管累积角膜的圆周钟点数，L 为新生血管从角膜缘伸入角膜的长度，R 为受试动物角膜平均半径（本次实验为 3mm），结果见图 5。

[0092] 结果显示：术后 15 天，融合蛋白 FP1、FP3 与免疫抑制剂（雷帕霉素、地塞米松、环孢素 A）联合用药，对角膜血管新生抑制具有协同作用（$p < 0.05$），并且，FP3、FP3 与免疫抑制剂的联合用药对角膜血管新生抑制作用最强，角膜水肿程度较轻，新生血管较细小并分布稀疏。

序列表

<110> 成都康弘生物科技有限公司

<120>VEGF 受体融合蛋白在制备治疗眼表血管新生疾病的药物中的应用

<130> 无

<160>4

<170>PatentIn version 3.5

<210>1

<211>455

<212>PRT

<213> 人工序列

<400>1

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
1 5 10 15
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu
20 25 30
Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu
35 40 45
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu
50 55 60
Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
65 70 75 80
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu
85 90 95
Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys
100 105 110
Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val
115 120 125
Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val
130 135 140
Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn
145 150 155 160
Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg
165 170 175
Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr
180 185 190
Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys
195 200 205
Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg

210 215 220

Val His Glu Lys Asp Lys Thr His Thr Cys Pro Leu Cys Pro Ala Pro
225 230 235 240

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400

Ala Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445

Leu Ser Leu Ser Pro Gly Lys
450 455

<210>2

<211>552

<212>PRT

<213> 人工序列

<400>2

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
1 5 10 15

Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu

20 25 30

Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu

35 40 45

Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu

50 55 60

Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile

65 70 75 80

Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu

85 90 95

Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys

100 105 110

Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val

115 120 125

Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val

130 135 140

Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn

145 150 155 160

Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg

165 170 175

Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr

180 185 190

Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys

195 200 205

Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg

210 215 220

Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu

225 230 235 240

Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Ala Lys Tyr Leu

245 250 255

Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Leu

260 265 270

Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr Ile Met Glu

275 280 285

Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro

290 295 300

Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val Val Tyr Val

305 310 315 320

Pro Pro Gly Pro Gly Asp Lys Thr His Thr Cys Pro Leu Cys Pro Ala

325 330 335

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
340 345 350
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
355 360 365
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
370 375 380
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
385 390 395 400
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
405 410 415
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
420 425 430
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
435 440 445
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
450 455 460
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
465 470 475 480
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
485 490 495
Lys Ala Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
500 505 510
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
515 520 525
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
530 535 540
Ser Leu Ser Leu Ser Pro Gly Lys
545 550

<210>3
<211>308
<212>PRT
<213> 人工序列
<400>3
Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His
1 5 10 15
Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro
20 25 30
Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro

35 40 45

Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser
50 55 60

Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val
65 70 75 80

Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn
85 90 95

Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser
100 105 110

Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn
115 120 125

Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His
130 135 140

Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met
145 150 155 160

Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp
165 170 175

Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys
180 185 190

Asn Ser Thr Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly
195 200 205

Ser Gly Met Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg
210 215 220

Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr
225 230 235 240

Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His
245 250 255

Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr
260 265 270

Val Ile Leu Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val
275 280 285

Ser Leu Val Val Tyr Val Pro Pro Asp Lys Thr His Thr Cys Pro Leu
290 295 300

Cys Pro Ala Pro
305

<210>4
<211>214
<212>PRT

<213> 人工序列

<400>4

```
Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His
1               5                  10                  15
Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro
            20                  25                  30
Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro
        35                  40                  45
Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser
    50                  55                  60
Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val
65                  70                  75                  80
Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn
                85                  90                  95
Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser
            100                 105                 110
Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn
        115                 120                 125
Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His
    130                 135                 140
Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met
145                 150                 155                 160
Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp
                165                 170                 175
Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys
            180                 185                 190
Asn Ser Thr Phe Val Arg Val His Glu Lys Asp Lys Thr His Thr Cys
        195                 200                 205
Pro Leu Cys Pro Ala Pro
    210
```

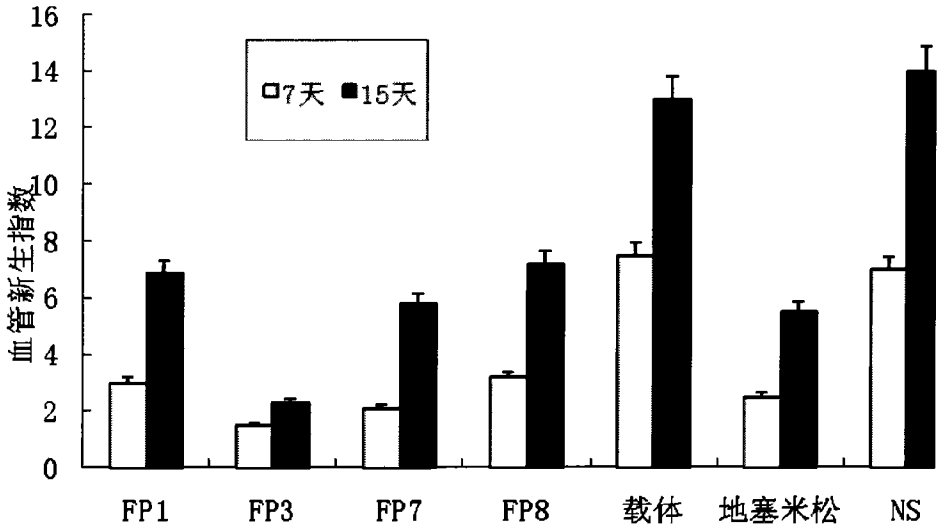


图 1

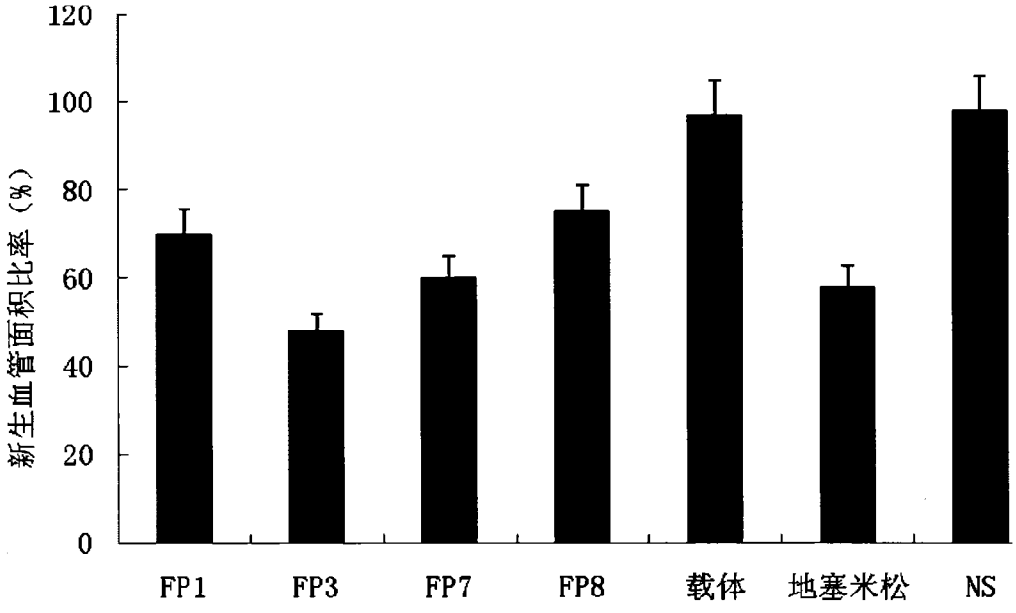


图 2

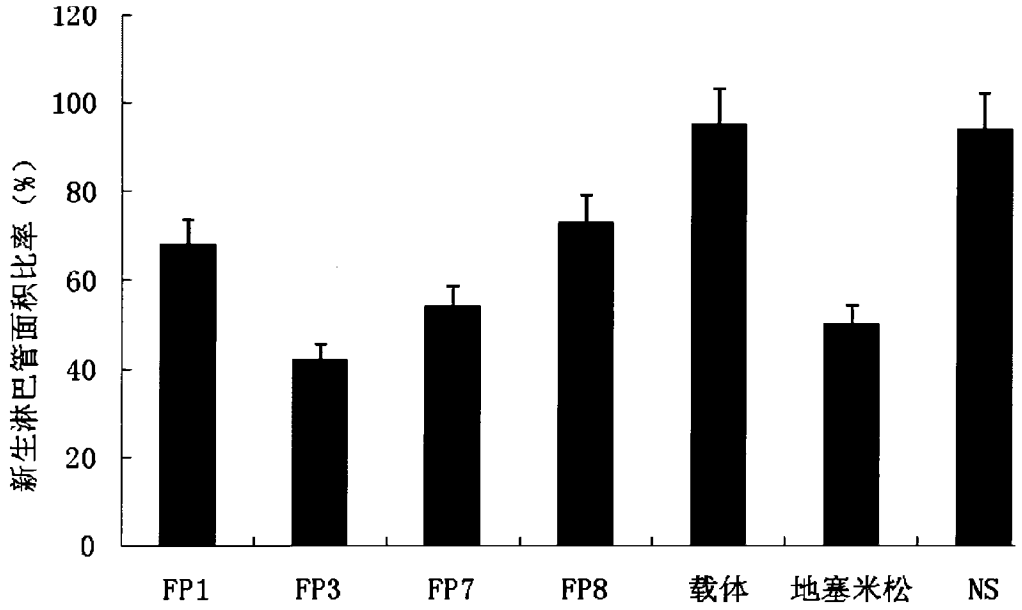


图 3

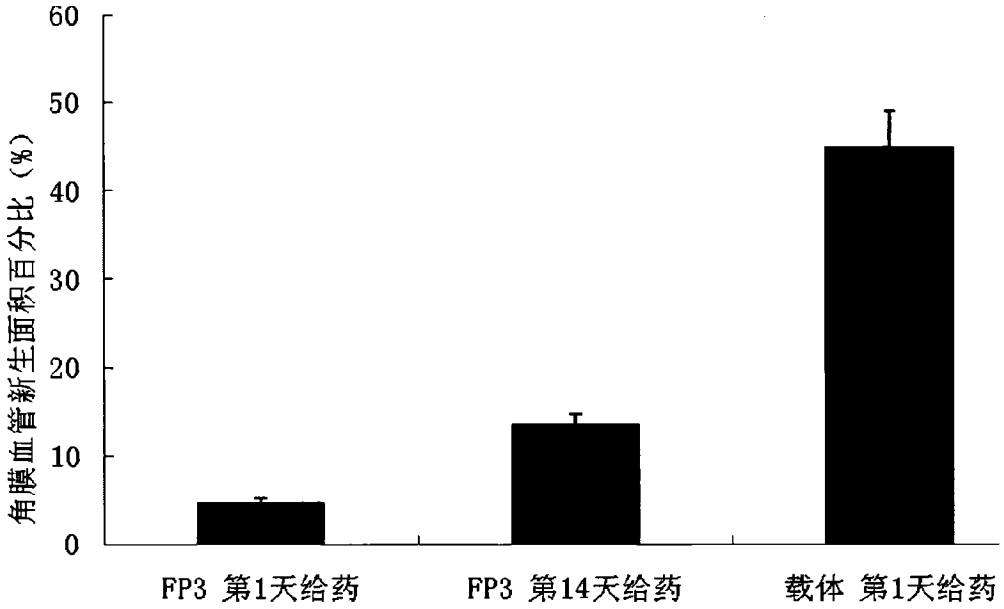


图 4

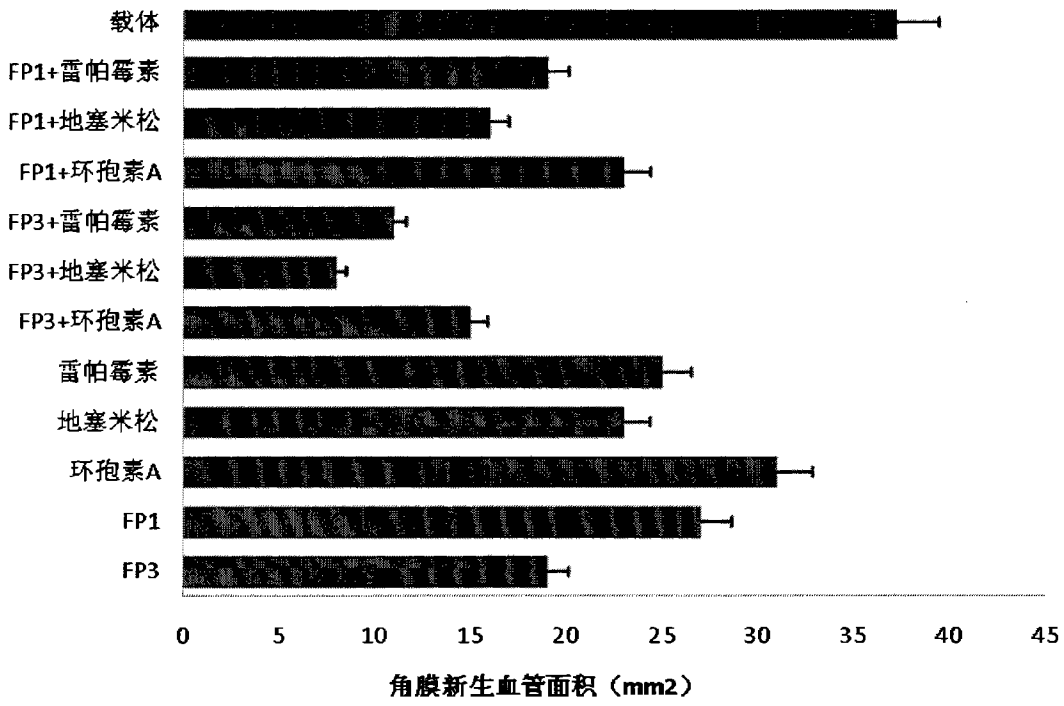


图 5

Notice

ABSTRACT CN102380096B

[0001]

13 The invention discloses a pharmaceutical composition containing a fusion protein that inhibits vascular proliferation and its use, and specifically relates to a containing extracellular domain 2 (Flt-2) of vascular endothelial cell growth factor (VEGF) receptor 1 and VEGF A pharmaceutical composition of the fusion protein of the extracellular domains 3 and 4 (KDR-3, 4) of receptor 2 and human immunoglobulin 1 (G1) Fc. The pharmaceutical composition can keep the fusion protein stable, the most prominent The characteristic is that it can effectively inhibit the decrease in purity caused by the production of fusion protein polymers, thereby maintaining the biological activity of the effective components.

CN102380096B Medicine combination containing fusion protein for suppressing angiogenesis and application

Technical field

The present invention relates to the field of pharmaceutical preparations, and to pharmaceutical compositions comprising a fusion of a VEGF receptor fragment and an immunoglobulin Fc, and their use in medicine.

Background technique

With the development of modern cell molecular biology, the role of cytokines and cell surface related molecules in ophthalmic diseases has been widely studied and studied. VEGF is a biological characteristic of vascular endothelial cells promoting mitogens to increase vascular permeability. During the fetal period, VEGF is extremely important for the occurrence of blood vessels, and the level after birth decreases. VEGF is in a low level of expression under physiological conditions and is essential for maintaining blood vessel function. The latest findings suggest a broad and important role in angiogenesis-related diseases such as age-related macular degeneration (AMD) and Diabetic retinopathy (DR). AMD occurs more than 45 years old, and its prevalence increases with age, which is an important disease for blindness in middle-aged and elderly people. Wet AMD is mainly the destruction of the vitreous membrane. The choroidal blood vessels invade the subretinal to form the choroidal neovascularization, and the macular area of the retinal pigment epithelium or the neuroepithelial serous or hemorrhagic discoid detachment eventually becomes a mechanical scar, which effectively inhibits the wetness. Macular endothelial growth factor VEGF, a macular degeneration, has an important therapeutic effect in blocking VEGF or VEGF receptors and inhibiting angiogenesis. In DR, the levels of VEGF in cells and body fluids are higher than normal. Increased VEGF, causing changes in capillary permeability, causing retinal exudation, hemorrhage and macular edema, induction of angiopoietin (Angiogenin) production, synergistically promote the formation of retinal neovascularization, resulting in visual impairment.

Salt bonds, hydrogen bonds, disulfide bonds, and hydrophobic interactions are mechanisms to maintain protein conformational stability. The interaction of metal ions, substrates, cofactors, and other low relative molecular weight ligands stabilizes the conformation of the protein. The action of proteins with other biological macromolecules, especially proteins and lipids. In organisms, proteins often interact with lipids or polysaccharides to form complexes that shield the hydrophobic regions of the protein surface, thereby significantly increasing protein stability. Protein instability is mainly caused by the following factors: (1) Physical effects: The contact of polar water molecules regulated by Brownian motion with the hydrophobic core of the protein leads to protein instability. (2) Chemical action: Oxidation of amino acid residues at the active site is one of the most common mechanisms of enzyme inactivation. For example, the sulfhydryl group of cysteine and the anthracene ring of tryptophan are particularly sensitive to oxidation. (3) Biological effects: protein hydrolase action. Microbial and exogenous proteolytic enzymes catalyze the hydrolysis of peptide bonds. The low yield of purified eukaryotic polypeptides from genetically engineered bacteria is due to in vitro proteolysis.

The polymerization first exposes the embedded hydrophobic amino acid residue to the aqueous solvent, resulting in protein reversibility; secondly, the protein molecules associate with each other to reduce the unfavorable exposure of the hydrophobic amino acid; finally, if the protein molecule contains cysteine and cysteine For the amino acid residue, an intermolecular disulfide exchange reaction occurs. Polymerization sometimes reactivates the protein by regenerating and reoxidizing the natural disulfide bond. There is a difference between polymerization and simple precipitation, which does not cause significant conformational changes in the protein.

The inventors have also observed that an obvious problem is that the solution preparation easily forms soluble polymers and insoluble particles after long-term storage. How to solve this problem and find a physically and chemically stable pharmaceutical composition capable of suppressing The polymer is formed and is capable of forming less soluble and insoluble particles after long term storage. In addition, since its components are pharmaceutically acceptable components, it can be used for the treatment of ocular diseases, including

intravitreal injection and external administration. Furthermore, the inventors have found that the resulting formulation formulation is more stable in a syringe than in a vial.

In the present invention, extracellular domains 3 and 4 (KDR-3, which are capable of inhibiting angiogenesis, from extracellular domain 2 (Flt-2) of vascular endothelial growth factor (VEGF) receptor 1 and VEGF receptor 2 are employed. 4) The fusion protein with human immunoglobulin 1 (G1) Fc (FP3 protein) is expressed by working cells of recombinant technology and purified to obtain medicinal purity, and the appropriate drug is prepared by substituting the liquid replacement preparation. preparation. These preparations are preferably liquid preparations or lyophilized preparations, and are suitable for the treatment of ocular diseases, especially for vitreous injection.

As a protein drug, FP3 protein is much less stable than ordinary small molecule chemical drugs, and its stability is also worse than that of naturally occurring immunoglobulins. The fusion protein described in the Chinese patent is the Chinese patent "Application of VEGF Receptor Fusion Protein in the Treatment of Eye Diseases" (Patent No. ZL200610066257.2). The prescription needs to be stored at -20 ° C for the production of drugs, transport preservation and Applications have put forward higher requirements. It is well known that the stability of the general recombinant fusion protein is relatively poor, and it is affected by various environmental factors during the preservation process. For example, temperature, moderateness, oxygen, ultraviolet light, etc. may be various physical or chemical changes of the fusion protein, resulting in Polymerization, decomposition, oxidation or denaturation of proteins. These changes can reduce the activity of the protein, reduce the therapeutic effect and cause serious side effects. Therefore, it has been of great clinical significance to develop a fusion protein preparation that is stable and easy to transport and store.

Summary of the invention

One of the objects of the present invention is to provide an extracellular domain 2 (Flt-2) containing vascular endothelial growth factor (VEGF) receptor 1 and extracellular domains 3 and 4 of VEGF receptor 2 (KDR- 3, 4) a pharmaceutical composition of a fusion protein with human immunoglobulin 1 (G1) Fc, more specifically a liquid preparation which can be used for vitreous injection, which can stabilize the fusion protein, its most prominent feature It is effective in inhibiting the decrease in purity caused by the production of the fusion protein polymer, thereby maintaining the biological activity of the effective component.

In one aspect, the present invention provides a pharmaceutical composition comprising a fusion protein that inhibits vascular proliferation, characterized by comprising

(a) 0.1-100 mg/ml of the extracellular domain 2 of VEGF receptor 1 and the extracellular domain 3 and 4 of VEGF receptor 2 and a fusion protein of human immunoglobulin Fc, comprising the amino acid of SEQ ID No: 1. sequence;

(b) 5-100 mM buffer, wherein the acid is selected from one or more of Tris-HCl, citric acid, sodium hydrogen phosphate, sodium dihydrogen phosphate, acetic acid, succinic acid, hydrochloric acid;

(c) a 5-500 mM basic amino acid selected from the group consisting of lysine, arginine, and histidine, or a combination thereof;

(d) 0.1-30% salt osmotic pressure regulator, wherein the sugar is selected from one or more of sucrose, trehalose, mannitol, glycerin, propylene glycol, sorbitol, the salt is selected from sodium chloride or other One or a combination of pharmaceutically acceptable salts;

(e) 0.01-0.1% of one or more surfactants or cosolvents selected from the group consisting of polyethylene glycol, Tween 20, Tween 80, propylene glycol, dimethyl sulfoxide or other pharmaceutically acceptable surface One or more of the active agents;

(f) The pH is adjusted to 7.5 to 8.3.

Wherein the pharmaceutical composition is preferably a pharmaceutical composition comprising the following components:

(a) a fusion protein of SEQ ID No: 1 of 10-40 mg/ml;

(b) one or both of 5-100 mM citric acid or sodium dihydrogen phosphate;

(c) one or two of 5-500 mM arginine or histidine;

(d) one or two of 8-30% of sucrose or trehalose;

(e) one or two of 0.01-0.1% of Tween-20 or polyethylene glycol;

(f) The pH is adjusted to 7.5 to 8.3.

Preferred pharmaceutical compositions of the invention may also contain sodium chloride.

More preferably, the present invention provides a fusion protein of SEQ ID No: 1, 10 mM citric acid, 5% sucrose, 100 mM arginine, 0.05% Tween 20, pH 7 from 10-40 mg/ml. A pharmaceutical composition consisting of .5 to 8.3.

The present invention still further provides a fusion protein of SEQ ID No: 1 of 10-40 mg/ml, 55 mM citric acid, 12.5% sucrose, 250 mM arginine, 0.05% Tween 20, pH 7.9. Buffer, pH 7.5 ~ 8.3.

The pharmaceutical composition prepared by the above pharmaceutical composition may be in the form of a liquid preparation or a lyophilized preparation, wherein the liquid preparation is preferably an ophthalmic preparation, especially an eye drop; or a pre-filled injection.

The above pharmaceutical composition can be used for the treatment of diseases caused by angiogenesis or growth, preferably age-related macular degeneration.

In the present invention, the fusion protein is a fusion protein described in the Chinese patent "Application of a VEGF receptor fusion protein in the treatment of ocular diseases" (Patent No. ZL200610066257.2), specifically a FP3 fusion protein, and thus ZL200610066257 The contents of .2 can be used to further illustrate the invention.

Detailed ways

Example 1 Stability of 10 mg/ml FP3 fusion protein in 3 ml glass ampoules at 4 ° C

The prescription is as follows:

FP3 fusion protein 10mg/ml

Sodium succinate 10 mM

Trehalose 9.0%

Tween 20 0.05%

Adjust the pH of the system to 6.0-6 with hydrochloric acid.5

After the protein stock solution was changed, it was aseptically dispensed into 3 ml glass ampoules, and samples were taken at 4 ° C, and determined at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and December. Samples were determined for stability by SEC-HPLC. The results show that the prescription can not effectively inhibit the formation of polymer, resulting in decreased product purity, decreased affinity with VEGF, and may induce an immune response after entering the body.

Table 1. Stability of 10 mg/ml FP3 fusion protein at 4 ° C

Time (month) Appearance pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 6.0 10.0 0.3 10.6 1 Qualified 6.0 9.4 1.9 9.5 2 Qualified 6.0 10.2 2.8 9.1 3 Qualified 6.0 10.2 3.6 8.7 6 Qualified 6.0 10.0 9.1 8.4 9 Qualified 6.0 10.0 18.2 7.7 12 Qualified 6.1 10.3 31.8 7.0

Example 2 Stability of 10 mg/ml FP3 fusion protein in 3 ml glass ampoules at 4 °C

FP3 fusion protein 10mg/ml

Disodium hydrogen phosphate 10 mM

Sucrose 10%

Sodium chloride 0.5%

Tween 20 0.05%

pH 7.5~8.3

After the protein stock solution was changed, it was aseptically dispensed into 3 ml glass ampoules, and samples were taken at 4 ° C, and determined at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and December. Samples were determined for stability by SEC-HPLC. The results show that the prescription can not effectively inhibit the formation of polymer, resulting in decreased product purity, decreased affinity with VEGF, and may induce an immune response after entering the body.

Table 2. Stability of 10 mg/ml FP3 fusion protein at 4 ° C

Time (month) Appearance pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 7.7 10.1 0.2 10.7 1 Qualified 7.9 10.1 2.0 9.9 2 Qualified 7.7 10.7 3.2 9.3 3 Qualified 7.8 10.6 5.4 8.4 6 Qualified 7.7 10.3 9.9 8.1 9 Qualified 7.5 10.1 13.1 8.5 12 Qualified 7.9 10.3 23.3 7.3

Example 3 Stability of 10 mg/ml FP3 fusion protein in 3 ml glass ampoules at 4 °C

FP3 fusion protein 10mg/ml

Citric acid 5mM

Sucrose 8.0%

Tween 20 0.05%

pH 7.5~8.3

After the protein stock solution was changed, it was aseptically dispensed into 3 ml glass ampoules, and samples were taken at 4 ° C, and determined at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and December. Samples were determined for stability by SEC-HPLC. The results show that the prescription can not effectively inhibit the formation of polymer, resulting in decreased product purity, decreased affinity with VEGF, and may induce an immune response after entering the body.

Table 3. Stability of 10 mg/ml FP3 fusion protein at 4 ° C

Time (month) Appearance pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 7.9 10.2 0.3 10.4 1 Qualified 7.9 10.2 1.4 10.0 2 Qualified 7.8 10.6 2.6 9.7 3 Qualified 7.8 10.7 3.6 8.1 6 Qualified 8.1 10.5 9.4 7.9 9 Qualified 8.3 10.1 18.5 9.7 12 Qualified 8.0 10.4 19.1 9.6

Example 4 Stability of 10 mg/ml FP3 fusion protein in 3 ml glass ampoules at 4 °C

FP3 fusion protein 10mg/ml

Citric acid 10mM

Sucrose 8.0%

Arginine 5mM

Tween 20 0.05%

pH 7.5~8.3

After the protein stock solution was changed, it was aseptically dispensed into 3 ml glass ampoules, and samples were taken at 4 ° C, and determined at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and December. Samples were determined for stability by SEC-HPLC. The results show that the prescription can effectively inhibit the formation of polymer, the purity of the product decreases slowly, and the affinity of the fusion protein to VEGF is almost unchanged.

Table 4. Stability of 10 mg/ml FP3 fusion protein at 4 °C

Time (month) Appearance pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 7.7 10.1 0.4 10.5 1 Qualified 7.9 10.1 0.5 10.4 2 Qualified 7.5 10.7 0.7 10.1 3 Qualified 7.8 10.6 0.8 10.0 6 Qualified 7.7 10.3 0.9 9.9 9 Qualified 7.9 10.1 1.8 9.7 12 Qualified 7.9 10.3 1.9 9.6

Example 5 Stability of 10 mg/ml FP3 fusion protein in 3 ml glass ampoules at 4 °C

FP3 fusion protein 10mg/ml

Citric acid 100mM

Sucrose 20.0%

Arginine 250mM

Tween 20 0.10%

pH 7.5~8.3

After the protein stock solution was changed, it was aseptically dispensed into 3 ml glass ampoules, and samples were taken at 4 ° C, and determined at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and December. Samples were determined for stability by SEC-HPLC. The results show that the prescription can effectively inhibit the formation of polymer, the purity of the product decreases slowly, and the affinity of the fusion protein to VEGF is almost unchanged.

Table 5. Stability of 10 mg/ml FP3 fusion protein at 4 °C

Time (month) Appearance pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 7.9 10.0 0.3 10.4 1 Qualified 7.9 10.1 0.3 10.3 2 Qualified 7.9 10.0 0.4 10.4 3 Qualified 7.9 10.1 0.5 10.2 6 Qualified 7.9 10.2 0.7 10.1 9 Qualified 7.9 10.0 0.8 10.2 12 Qualified 7.9 10.1 0.9 10.0

Example 6 Stability of 10 mg/ml FP3 fusion protein in 3 ml glass ampoules at 4 °C

FP3 fusion protein 10mg/ml

Sodium dihydrogen phosphate 5 mM

Trehalose 10.0%

Arginine 100mM

PEG400 0.01%

pH 7.5~8.3

After the protein stock solution was changed, it was aseptically dispensed into 3 ml glass ampoules, and samples were taken at 4 ° C, and determined at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and December. Samples were determined for stability by SEC-HPLC.

Table 6. Stability of 10 mg/ml FP3 fusion protein at 4 °C

Example 7 Stability of 20 mg/ml FP3 fusion protein in 3 ml glass ampoules at 4 °C

FP3 fusion protein 20mg/ml

Citric acid 5mM

Sucrose 4.0%

Sodium chloride 4.0%

Arginine 100mM

Histidine 100 mM

Tween 20 0.05%

PEG400 0.05%

pH 7.5~8.3

After the protein stock solution was changed, it was aseptically dispensed into 3 ml glass ampoules, and samples were taken at 4 ° C, and determined at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and December. Samples were determined for stability by SEC-HPLC.

Table 7.20 mg/ml FP3 fusion protein stability at 4 ° C

Time (month) Appearance pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 8.0 19.8 1 14.3 1 Qualified 7.8 20.7 1.5 10.2 2 Qualified 7.9 19.8 1.3 12.6 3 Qualified 7.9 20.6 2 10.5 4 Qualified 7.8 N /D 1.7 N/D 5 Qualified 7.8 N/D 2.2 N/D 6 Qualified 8.0 N/D 2.7 N/D 7 Qualified 7.8 N/D 2.7 N/D 8 Qualified 7.9 N/D 2.7 N/D 9 Qualified 7.9 19.5 2.7 12.5 10 Qualified 7.8 N/D 3.1 N/D 11 Qualified 7.8 N/D 3.5 N/D 12 Qualified 7.9 20.3 3.6 14.3

N/D means not detected.

Example 8 Stability of 20 mg/ml FP3 fusion protein in 3 ml glass ampoules at 4 °C

FP3 fusion protein 20mg/ml

Citric acid 10mM

Sucrose 30%

Arginine 500M

Tween 20 0.1%

pH 7.5~8.3

After the protein stock solution was changed, it was aseptically dispensed into 3 ml glass ampoules, and samples were taken at 4 ° C, and determined at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and December. Samples were determined for stability by SEC-HPLC.

Table 8.20 mg/ml FP3 fusion protein stability at 4 ° C

Time (month) Appearance pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 7.7 20.0 0.1 10.2 1 Qualified 7.9 20.0 0.1 10.2 2 Qualified 7.7 20.2 0.1 10.3 3 Qualified 7.8 20.2 0.2 10.3 6 Qualified 7.7 20.1 0.2 9.9 9 Qualified 7.9 20.3 0.3 10.0 12 Qualified 7.9 20.0 0.5 10.3

Example 9 Stability of 10 mg/ml FP3 fusion protein in 3 ml glass ampoules at 4 °C

FP3 fusion protein 10mg/ml

Citric acid 10mM

Sucrose 5%

Arginine 100M

Tween 20 0.05%

pH 7.5~8.3

After the protein stock solution was changed, it was aseptically dispensed into 3 ml glass ampoules, and samples were taken at 4 ° C, and determined at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and December. Samples were determined for stability by SEC-HPLC.

Table 9. Stability of 10 mg/ml FP3 fusion protein at 4 ° C

Time (month) Appearance pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 7.7 10.1 0.4 10.5 1 Qualified 7.9 10.1 0.5 10.4 2 Qualified 7.7 10.7 0.7 10.1 3 Qualified 7.8 10.6 0.8 10.0 6 Qualified 7.7 10.3 0.9 9.9 9 Qualified 7.9 10.1 1.8 7.7 12 Qualified 7.9 10.3 1.9 6.3

Example 10 Stability of 10 mg/ml FP3 fusion protein in 3 ml glass ampoules at 4 °C

The FP3 fusion protein stock solution was aliquoted by Vivaflow and then aseptically divided into semi-finished products, 55 mM citric acid, 12.5% sucrose, 250 mM arginine, 0.05% Tween 20, pH 7.9 buffer. Adjust the FP3 fusion protein to 10mg/ml, aseptically to obtain 3ml glass ampoule, and store at 4°C, at 0,1,2,3,4,5,6,7,8,9,10,11 The samples were assayed in December and stability was determined by SEC-HPLC.

Table 10. Stability of 10 mg/ml FP3 fusion protein at 4 ° C

Time (month) Appearance pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 8.3 10.0 0.2 9.7 1 Qualified 8.3 10.1 0.2 9.6 2 Qualified 8.2 10.2 0.3 9.6 3 Qualified 7.9 10.0 0.4 9.3 6 Qualified 8.0 10.1 0.5 9.3 9 Qualified 7.9 10.1 0.6 9.1 12 Qualified 7.9 10.3 1.0 9.4

Example 11 Stability of 20 mg/ml FP3 fusion protein in 3 ml glass ampoules at 4 °C

The FP3 fusion protein stock solution was aliquoted by Vivaflow and then aseptically divided into semi-finished products, 55 mM citric acid, 12.5% sucrose, 250 mM arginine, 0.05% Tween 20, pH 7.5-8.3. Buffer, FP3 fusion protein was adjusted to 20 mg/ml, aseptically dispensed into 3 ml glass ampoules, and sampled at 4 °C, at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 The samples were assayed at November and December and stability was determined by SEC-HPLC.

Table 11. Stability of the 20 mg/ml FP3 fusion protein at 4 ° C

Time (month) Appearance pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 7.9 19.9 0.7 10.7 1 Qualified 7.9 20.1 1.2 10.2 2 Qualified 7.7 20.7 1.3 10.3 3 Qualified 7.8 21.6 1.4 10.4 6 Qualified 8.0 21.6 1.4 10.4 9 Qualified 7.9 21.1 2.6 9.6 12 Qualified 7.9 21.3 3.6 9.4

Example 12 Stability of 40 mg/ml FP3 fusion protein lyophilized preparation at 4 ° C

FP3 fusion protein 40mg/ml

Citric acid 250mM

Sucrose 8.0%

Histidine 100 mM

Tween 20 0.10%

pH 7.5~8.3

The FP3 fusion protein solution was adjusted to 40 mg/ml, the pH was adjusted to 7.5-8.3, and the mixture was dispensed into 3 ml glass ampoules. The optimized lyophilization curve was: pre-freezing for 4 hours at -50 °C, controlled at Sublimation at -20 °C removes most of the water, then gradually raises the temperature of the separator to further remove residual moisture. The final step is to apply 25 degrees and reach the ultimate vacuum to lower the moisture while keeping the temperature of the sample not too high. After lyophilization, Yasai, lyophilized box, rolled aluminum cover, analysis of water and purity, sample at 4 °C, re-dissolve in equal volume of water for injection at 0,1,6,12 months, determine the sample, pass Stability was determined by SEC-HPLC.

Table 12. Stability of 40 mg/ml FP3 fusion protein at 4 °C

Time (month) Appearance after reconstitution pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 7.9 39.9 0.1 11.2 1 Qualified 7.9 40.1 0.2 10.6 6 Qualified 7.9 40.7 0.3 10.1 12 Qualified 8.3 40.6 0.4 10.0

Example 13 Stability of 40 mg/ml FP3 fusion protein lyophilized preparation at 4 ° C

FP3 fusion protein 40mg/ml

Citric acid 10mM

Sucrose 5%

Arginine 100mM

Tween 20 0.05%

pH 7.5~8.3

The FP3 fusion protein solution was adjusted to 20 mg/ml, the pH was adjusted to 7.5-8.3, and the mixture was dispensed into 3 ml glass ampoules. The optimized lyophilization curve conditions were: -50 °C rapid pre-freezing for 4 hours, controlled at Sublimation at -20 °C removes most of the water, then gradually raises the temperature of the separator to further remove residual moisture. The final step is to apply 25 degrees and reach the ultimate vacuum to lower the moisture while keeping the temperature of the sample not too high. After lyophilization, Yasai, lyophilized box, rolled aluminum cover, analysis of water and purity, sample at 4 °C, re-dissolve in equal volume of water for injection at 0,1,6,12 months, determine the sample, pass Stability was determined by SEC-HPLC.

Table 13. Stability of 40 mg/ml FP3 fusion protein at 4 ° C

Time (month) Appearance after reconstitution pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 8.0 40 0.1 10.2 1 Qualified 8.3 40 0.1 10.5 6 Qualified 7.9 40 0.2 10.2 12 Qualified 8.2 40.1 0.3 10.3

Example 14 Stability of 20 mg/ml FP3 fusion protein in glass prefilled syringe at 4 °C

FP3 fusion protein 20mg/ml

Citric acid 55mM

Sucrose 12.5%

Arginine 250mM

Tween 20 0.05%

pH 7.5~8.3

The FP3 fusion protein stock solution was aseptically divided into a semi-finished product, and the FP3 fusion protein was adjusted to 20 mg/ml, and aseptically dispensed into a 1 ml glass glass prefilled syringe with FluroTec coating at 4 ° C. Samples were taken and samples were measured at 0, 1, 6, and 12 months, and stability was determined by SEC-HPLC.

Stability of 20mg/ml FP3 fusion protein in glass pre-filled syringe at 14.4 °C

Time (month) Appearance pH Concentration (mg/ml) Polymer (%) Affinity (pM) 0 Qualified 7.9 20.0 0.1 11.2 1 Qualified 7.9 20.1 0.1 11.6 6 Qualified 7.9 20.1 0.1 11.1 12 Qualified 8.2 20.1 0.1 11.0

Example 15 Gel Exclusion Chromatography Analysis of Polymers (SEC-HPLC)

The porous silica gel column has high stability, heat resistance and pressure resistance, long life, and can realize rapid separation of biological macromolecules. TSK G3000SWxl is a porous silica gel column with a particle size of 5μm, a pore size of 250 angstroms, a separation globulin range of 10-500kD, and a sample separation time of 30 minutes. It is an internationally used analytical column for the use of biological macromolecules. In the present invention, we use a porous silica gel column TSKG3000SWxl column, the instrument is a 2695 high performance liquid chromatograph of Waters, with a pH of 7.20 phosphate buffer as a mobile phase, a flow rate of 0.5 ml/ml, a column temperature of 25 ° C, The detection wavelength was 280 nm, and the purity of the recombinant human vascular endothelial growth factor receptor-antibody FP3 fusion protein was examined.

Example 16 Rabbit vitreous injection repeated administration test

12Only Japanese white rabbits were randomly divided into two groups, the test group and the solvent control group, with 6 rats in each group, half male and half female. In the right eye of each group of animals, a single intravitreal injection was given to the test article (0.5 mg / 50 μL / eye) or an equal volume of solvent control, and the other side was used as a sham injection control or blank control. Visual observation was performed daily after administration, and indirect ophthalmoscopes and slit lamps were used for regular examination. The animals were sacrificed 14 days after administration, and the eyeballs were taken for histopathological examination. The local toxicity of the test article was examined by observation and histopathological examination results. The results showed that: the eyes of Japanese white rabbits were injected intravitreally, and 6 eyes were given to the testicles. One eye was observed to have slight congestion, and one was found to have a small amount of secretions. The slit lamp was observed to have a lens. turbid. At the same time, the same phenomenon was observed in the eyes of the control side, the sham injection side and the blank control side, respectively, and the incidence and the occurrence time were basically the same as those of the side to which the test article was administered. This eliminates the fact that these phenomena are irritating reactions caused by the test article. Histopathological examination showed that all eye tissue structures were normal. Therefore, under the conditions of this experiment, a single intravitreal injection of rabbit eyes was given recombinant human vascular endothelial growth factor receptor-antibody fusion protein injection without causing local irritation or tissue damage response.

CN102380096B Medicine combination containing fusion protein for suppressing angiogenesis and application

1.A pharmaceutical composition comprising a fusion protein for inhibiting angiogenesis, characterized by consisting of 10-40 mg/ml of the fusion protein as shown in SEQ ID No: 1, 10 mM citrate buffer, 5% Sucrose, 100 mM arginine, 0.05% Tween 20, pH 7.5-8.3.

2.A pharmaceutical composition comprising a fusion protein for inhibiting angiogenesis, characterized by comprising: 10-40 mg/ml of the fusion protein as shown in SEQ ID No: 1, 55 mM citrate buffer, 12.5% Sucrose, 250 mM arginine, 0.05% Tween 20, pH 7.5-8.3.

3.The pharmaceutical composition according to any one of claims 1 to 2, wherein the fusion protein as SEQ ID No: 1 is 10 mg/ml.

4.The pharmaceutical composition according to any one of claims 1 or 2 is a liquid preparation.

5.The pharmaceutical composition according to any one of claims 1 or 2 is a lyophilized preparation.

6.The pharmaceutical composition according to claim 4, characterized in that the liquid preparation produced is an ophthalmic preparation.

7.The pharmaceutical composition according to claim 6, wherein the ophthalmic preparation is an eye drop.

8.The pharmaceutical composition according to claim 4, characterized in that the preparation form is a pre-filled injection.

9.The pharmaceutical composition according to any one of claims 1 to 8, which is for use in the preparation of a medicament for treating a disease caused by angiogenesis or growth.

10.The pharmaceutical composition according to claim 9, wherein the disease is age-related macular degeneration.

(19)中华人民共和国国家知识产权局

(12)发明专利

(10)授权公告号 CN 102380096 B
(45)授权公告日 2014.04.30

(21)申请号 201010267503.7

(22)申请日 2010.08.31

(73)专利权人 成都康弘生物科技有限公司
地址 610036 四川省成都市金牛区蜀西路36号

(72)发明人 柯潇 郑强

(51)Int.Cl.
A61K 39/395(2006.01)
A61K 47/34(2006.01)
A61P 27/02(2006.01)

(56)对比文件
CN 101397343 A,2009.04.01,
CN 1706867 A,2005.12.14, 全文.
CN 1915427 A,2007.02.21, 全文.
CN 101205252 A,2008.06.25,

审查员 韦炜

权利要求书1页 说明书12页
序列表3页

(54)发明名称

一种含有抑制血管增生的融合蛋白的药物组合物及用途

(57)摘要

本发明公开了一种含有抑制血管增生的融合蛋白的药物组合物及用途，具体涉及一种含有的血管内皮细胞生长因子(VEGF)受体1的细胞外结构域2(Flt-2)和VEGF受体2的细胞外结构域3和4(KDR-3,4)与人免疫球蛋白1(G1)Fc的融合蛋白的药物组合物，该药物组合物能使融合蛋白保持稳定，其最突出的特征是能有效抑制融合蛋白聚合物的产生而造成的纯度下降，从而保持有效组分的生物活性。

CN 102380096 B

1. 一种含有抑制血管增生的融合蛋白的药物组合物，其特征在于由以下组分组成：10-40mg / ml 的如 SEQ ID No :1 所示的融合蛋白、10mM 的柠檬酸缓冲液，5％的蔗糖，100mM 的精氨酸，0.05％的吐温 20，pH7.5 ～ 8.3。

2. 一种含有抑制血管增生的融合蛋白的药物组合物，其特征在于由以下组分组成：10-40mg / ml 的如 SEQ ID No :1 所示的融合蛋白、55mM 的柠檬酸缓冲液，12.5％的蔗糖，250mM 的精氨酸，0.05％的吐温 20，pH7.5 ～ 8.3。

3. 根据权利要求 1 或 2 中任一项所述的药物组合物，其特征在于所述的如 SEQ ID No : 1 的融合蛋白为 10mg / ml。

4. 权利要求 1 或 2 中任一项所述的药物组合物为液体制剂。

5. 权利要求 1 或 2 中任一项所述的药物组合物为冻干制剂。

6. 根据权利要求 4 所述的药物组合物，其特征在于制成的液体制剂为眼用制剂。

7. 根据权利要求 6 所述的药物组合物，其特征在于所述的眼用制剂为滴眼液。

8. 根据权利要求 4 所述的药物组合物，其特征在于制剂形式为预填充注射剂。

9. 根据权利要求 1-8 中任一项所述的药物组合物，其特征在于在制备治疗由血管新生或生长引起的疾病的药物中的用途。

10. 根据权利要求 9 所述的药物组合物，其特征在于所述的疾病为年龄相关性黄斑变性。

一种含有抑制血管增生的融合蛋白的药物组合物及用途

技术领域

[0001] 本发明涉及药物制剂领域，涉及包含 VEGF 受体片段和免疫球蛋白 Fc 融合而成的蛋白的药物组合物，以及其在医疗上的应用。

背景技术

[0002] 随着现代细胞分子生物学的发展，细胞因子和细胞表面相关分子在眼科疾病中的作用已经被广泛的关注和研究，VEGF 是血管内皮细胞促有丝分裂素具有增加血管通透性的生物特性，在胎儿时期 VEGF 对血管的发生极为重要，出生后水平下降。生理状态下 VEGF 呈低水平表达状态，对于维持血管的功能是必要的。最新的研究结果提示，对于老年性视网膜血管病变 (Age-related macular degeneration，简称为 AMD)、糖尿病视网膜病变 (Diabetic retinopathy，DR) 等血管新生相关的疾病都起着广泛和重要的作用。AMD 多发生于 45 岁以上，其患病率随年龄的增长而增高，是当前中老年人致盲的重要疾病。湿性 AMD 主要为玻璃膜的破坏，脉络膜血管侵入视网膜下构成脉络膜新生血管，发生黄斑区视网膜色素上皮下或神经上皮下浆液性或出血性的盘状脱离，最终成为机化瘢痕，有效抑制导致湿性黄斑病变的血管内皮细胞生长因子 VEGF，阻断 VEGF 或 VEGF 受体从而达到抑制血管新生具有重要的有着重要的治疗作用。在 DR 中，细胞和体液中 VEGF 的含量高于正常水平。VEGF 增高，引起毛细血管通透性改变，引起视网膜渗出，出血及视网膜黄斑水肿，诱导血管生成素 (Angiogenin) 生成增加，协同促进视网膜新生血管的形成，造成视力损害。

[0003] 盐键、氢键、二硫键和疏水作用是维持蛋白质构象稳定作用力。金属离子、底物、辅助因子和其他低相对分子量配体的相互作用使蛋白构象稳定。蛋白质与其它的生物大分子尤其是蛋白质与脂的作用。在生物体内，蛋白质常与脂类或多糖相互作用形成复合物，屏蔽了蛋白质表面的疏水区域，从而显著增加蛋白质的稳定性。蛋白不稳定主要由以下因素引起：(1) 物理作用：由布朗运动调节的极性水分子与蛋白质疏水核的接触会导致蛋白质不稳定。(2) 化学作用：活性部位的氨基酸残基的氧化作用是酶失活的最常见机理之一。如半胱氨酸的巯基和色氨酸的吲哚环，对氧化特别敏感。(3) 生物学作用：蛋白质水解酶作用。微生物和外源蛋白水解酶作用催化肽键水解。由基因工程菌纯化真核细胞多肽时收率低，是由于体外蛋白水解造成的。

[0004] 聚合作用首先使包埋的疏水性氨基酸残基暴露于水溶剂，导致蛋白质可逆变性；其次，蛋白质分子彼此缔合，以减少疏水氨基酸的不利裸露；最后，如果蛋白质分子含有半胱氨酸和胱氨酸残基，则会发生分子间二硫键交换反应。聚合作用有时可通过还原和再氧化再生天然二硫键，使蛋白质再活化。聚合和简单沉淀是有区别的，后者并未使蛋白质发生显著的构象变化。

[0005] 本发明人还观察到一个明显的问题是溶液制剂在长期储存后容易形成可溶聚合物和不可溶颗粒，如何解决这个问题，找到一种在物理和化学上都稳定的药物组合物，能够抑制聚合物生成，并能够在长期储存后形成较少的可溶聚合物和不溶颗粒。另外，由于其组分是药学上可接受的组分，可以用于治疗眼部疾病，包括玻璃体内注射和外部给药。此

外，本发明人发现得到的制剂处方在注射器中比在小瓶中更加稳定。

[0006] 本发明中采用能够抑制血管新生，由血管内皮细胞生长因子(VEGF)受体1的细胞外结构域2(Flt-2)和VEGF受体2的细胞外结构域3和4(KDR-3,4)与人免疫球蛋白1(G1)Fc的融合而成的蛋白(FP3蛋白)是通过重组技术的工作细胞表达并经过纯化后达到药用纯度，经过换液制剂分装制得合适的药物制剂。这些制剂优选液体制剂或冻干制剂，适用于眼睛疾病的治疗，尤其是用于玻璃体注射。

[0007] FP3蛋白作为蛋白药物，其稳定性比普通小分子化学药物差很多，相比于天然存在的免疫球蛋白，其稳定性也更差。中国专利所述的融合蛋白是中国专利“VEGF受体融合蛋白在治疗眼睛疾病中的应用”(专利号ZL200610066257.2)中描述的处方需要在-20℃保存，对药物的生产，运输保存和应用都提出了较高的要求。众所周知，一般重组的融合蛋白的稳定性都比较差，在保存过程中会受到多种环境因素的影响，如温度，适度，氧，紫外线等都可以是融合蛋白发生多种物理或化学变化，造成蛋白质的聚合，分解，氧化或变性等。这些变化都可以使蛋白的活性降低，治疗效果下降并引起严重的毒副作用。因此，开发出稳定且易于运输和贮藏的融合蛋白制剂是具有十分重要的临床意义的。

发明内容

[0008] 本发明的目的之一在于提供一种含有的血管内皮细胞生长因子(VEGF)受体1的细胞外结构域2(Flt-2)和VEGF受体2的细胞外结构域3和4(KDR-3,4)与人免疫球蛋白1(G1)Fc的融合蛋白的药物组合物，更具体的是能用于玻璃体注射的液体制剂，该液体制剂能使融合蛋白保持稳定，其最突出的特征是能有效抑制融合蛋白聚合物的产生而造成的纯度下降，从而保持有效组分的生物活性。

[0009] 本发明一方面提供了一种含有抑制血管增生的融合蛋白的药物组合物，其特征在于包含

[0010] (a)0.1-100mg/ml的VEGF受体1的细胞外结构域2和VEGF受体2的细胞外结构域3和4与人免疫球蛋白Fc的融合蛋白，包含SEQ ID No:1的氨基酸序列；

[0011] (b)5-100mM缓冲液，其中的酸选自Tris-HCl，柠檬酸，磷酸氢钠，磷酸二氢钠，醋酸，丁二酸，盐酸中的一种或多种；

[0012] (c)5-500mM碱性氨基酸选自赖氨酸，精氨酸，和组氨酸中的一种或其组合；

[0013] (d)0.1-30％盐渗透压剂调节剂，其中的糖选自蔗糖，海藻糖，甘露醇，甘油，丙二醇，山梨酯醇中的一种或多种，盐选自氯化钠或其它药学上可以接受的盐中的一种或其组合；

[0014] (e)0.01-0.1％的一种或多种表面活性剂或助溶剂，选自聚乙二醇，吐温20，吐温80，丙二醇，二甲基亚砜或其它药学上可以接受的表面活性剂中的一种或多种；

[0015] (f)调节pH为7.5～8.3。

[0016] 其中药物组合物优选为包含下列组分的药物组合物：

[0017] (a)10-40mg/ml的如SEQ ID No:1的融合蛋白；

[0018] (b)5-100mM的柠檬酸或磷酸二氢钠的一种或两种；

[0019] (c)5-500mM的精氨酸或组氨酸的一种或两种；

[0020] (d)8-30％的蔗糖或海藻糖的一种或两种；

[0021] (e)0.01-0.1%的吐温 -20 或聚乙二醇的一种或两种 ;

[0022] (f) 调节 pH 为 7.5 ～ 8.3。

[0023] 本发明所述优选的药物组合物还可以含有氯化钠。

[0024] 本发明更优的提供了一种由 10-40mg/ml 的如 SEQ ID No :1 的融合蛋白、10mM 的柠檬酸,5%的蔗糖,100mM 的精氨酸,0.05%的吐温 20,pH7.5 ～ 8.3 组成的药物组合物。

[0025] 本发明还进一步提供了一种 10-40mg/ml 的如 SEQ ID No :1 的融合蛋白、55mM 的柠檬酸,12.5%的蔗糖,250mM 的精氨酸,0.05%的吐温 20,pH7.9 缓冲液,pH7.5 ～ 8.3。

[0026] 上述药物组合物制成的制剂形式可为液体制剂或冻干制剂,其中液体制剂优选为眼用制剂,尤其是滴眼液 ;还可以为预填充注射剂。

[0027] 上述药物组合物可以用于治疗血管新生或生长引起的疾病,优选为年龄相关性黄斑变性。

[0028] 在本发明中,所述的融合蛋白是中国专利“VEGF 受体融合蛋白在治疗眼睛疾病中的应用”(专利号 ZL200610066257.2) 中描述的融合蛋白,具体而言是 FP3 融合蛋白,因此 ZL200610066257.2 的内容可用于进一步阐述本发明。

具体实施方式

[0029] 实施例 1 10mg/ml FP3 融合蛋白原处方在 4℃下 3ml 玻璃安瓿中的稳定性研究

[0030] 处方如下 :

[0031] FP3 融合蛋白 10mg/ml

[0032] 丁二酸钠 10mM

[0033] 海藻糖 9.0%

[0034] 吐温 20 0.05%

[0035] 用盐酸调节系统 pH 到 6.0 ～ 6.5

[0036] 将蛋白原液换液后,无菌分装得到 3ml 玻璃安瓿中,4℃留样,在 0,1,2,3,4,5,6,7,8,9,10,11,12 月测定样品,通过 SEC-HPLC 确定稳定性。结果显示,该处方不能有效抑制聚合物的生成,造成产品纯度下降,与 VEGF 的亲和力降低,进入体内以后可能诱发免疫反应。

[0037] 表 1.10mg/ml FP3 融合蛋白在 4℃的稳定性

[0038]

时间 (月)	外观	pH 值	浓度 (mg/ml)	聚合物 (%)	亲和力 (pM)
0	合格	6.0	10.0	0.3	10.6
1	合格	6.0	9.4	1.9	9.5
2	合格	6.0	10.2	2.8	9.1
3	合格	6.0	10.2	3.6	8.7
6	合格	6.0	10.0	9.1	8.4

9	合格	6.0	10.0	18.2	7.7
12	合格	6.1	10.3	31.8	7.0

[0039] 实施例 2 10mg/ml FP3 融合蛋白在 4℃下 3ml 玻璃安瓿中的稳定性研究

[0040] FP3 融合蛋白 10mg/ml

[0041] 磷酸氢二钠 10mM

[0042] 蔗糖 10％

[0043] 氯化钠 0.5％

[0044] 吐温 20 0.05％

[0045] pH 7.5 ～ 8.3

[0046] 将蛋白原液换液后，无菌分装得到 3ml 玻璃安瓿中，4℃留样，在 0，1，2，3，4，5，6，7，8，9，10，11，12 月测定样品，通过 SEC-HPLC 确定稳定性。结果显示，该处方不能有效抑制聚合物的生成，造成产品纯度下降，与 VEGF 的亲和力降低，进入体内以后可能诱发免疫反应。

[0047] 表 2. 10mg/ml FP3 融合蛋白在 4℃的稳定性

[0048]

时间（月）	外观	pH 值	浓度 (mg/ml)	聚合物（％）	亲和力 (pM)
0	合格	7.7	10.1	0.2	10.7
1	合格	7.9	10.1	2.0	9.9
2	合格	7.7	10.7	3.2	9.3
3	合格	7.8	10.6	5.4	8.4
6	合格	7.7	10.3	9.9	8.1
9	合格	7.5	10.1	13.1	8.5
12	合格	7.9	10.3	23.3	7.3

[0049] 实施例 3 10mg/ml FP3 融合蛋白在 4℃下 3ml 玻璃安瓿中的稳定性研究

[0050] FP3 融合蛋白 10mg/ml

[0051] 柠檬酸 5mM

[0052] 蔗糖 8.0％

[0053] 吐温 20 0.05％

[0054] pH 7.5 ～ 8.3

[0055] 将蛋白原液换液后，无菌分装得到 3ml 玻璃安瓿中，4℃留样，在 0，1，2，3，4，5，6，7，8，9，10，11，12 月测定样品，通过 SEC-HPLC 确定稳定性。结果显示，该处方不能有效抑制聚合物的生成，造成产品纯度下降，与 VEGF 的亲和力降低，进入体内以后可能诱发免疫反

应。

[0056] 表 3. 10mg/ml FP3 融合蛋白在 4℃的稳定性

[0057]

时间（月）	外观	pH 值	浓度 (mg/ml)	聚合物（%）	亲和力 (pM)
0	合格	7.9	10.2	0.3	10.4
1	合格	7.9	10.2	1.4	10.0
2	合格	7.8	10.6	2.6	9.7
3	合格	7.8	10.7	3.6	8.1
6	合格	8.1	10.5	9.4	7.9
9	合格	8.3	10.1	18.5	9.7
12	合格	8.0	10.4	19.1	9.6

[0058] 实施例 4 10mg/ml FP3 融合蛋白在 4℃下 3ml 玻璃安瓿中的稳定性研究

[0059] FP3 融合蛋白 10mg/ml

[0060] 柠檬酸 10mM

[0061] 蔗糖 8.0%

[0062] 精氨酸 5mM

[0063] 吐温 20 0.05%

[0064] pH 7.5 ～ 8.3

[0065] 将蛋白原液换液后，无菌分装得到 3ml 玻璃安瓿中，4℃留样，在 0，1，2，3，4，5，6，7，8，9，10，11，12 月测定样品，通过 SEC-HPLC 确定稳定性。结果显示，该处方能有效抑制聚合物的生成，产品纯度下降很慢，融合蛋白与 VEGF 的亲和力几乎不变。

[0066] 表 4. 10mg/ml FP3 融合蛋白在 4℃的稳定性

[0067]

时间（月）	外观	pH 值	浓度 (mg/ml)	聚合物（%）	亲和力 (pM)
0	合格	7.7	10.1	0.4	10.5
1	合格	7.9	10.1	0.5	10.4
2	合格	7.5	10.7	0.7	10.1
3	合格	7.8	10.6	0.8	10.0
6	合格	7.7	10.3	0.9	9.9

9	合格	7.9	10.1	1.8	9.7
12	合格	7.9	10.3	1.9	9.6

[0068] 实施例 5 10mg/ml FP3 融合蛋白在 4℃下 3ml 玻璃安瓿中的稳定性研究

[0069] FP3 融合蛋白 10mg/ml

[0070] 柠檬酸 100mM

[0071] 蔗糖 20.0%

[0072] 精氨酸 250mM

[0073] 吐温 20 0.10%

[0074] pH 7.5 ～ 8.3

[0075] 将蛋白原液换液后，无菌分装得到 3ml 玻璃安瓿中，4℃留样，在 0，1，2，3，4，5，6，7，8，9，10，11，12 月测定样品，通过 SEC-HPLC 确定稳定性。结果显示，该处方能有效抑制聚合物的生成，产品纯度下降很慢，融合蛋白与 VEGF 的亲和力几乎不变。

[0076] 表 5. 10mg/ml FP3 融合蛋白在 4℃的稳定性

[0077]

时间（月）	外观	pH 值	浓度 (mg/ml)	聚合物（%）	亲和力 (pM)
0	合格	7.9	10.0	0.3	10.4
1	合格	7.9	10.1	0.3	10.3
2	合格	7.9	10.0	0.4	10.4
3	合格	7.9	10.1	0.5	10.2
6	合格	7.9	10.2	0.7	10.1
9	合格	7.9	10.0	0.8	10.2
12	合格	7.9	10.1	0.9	10.0

[0078] 实施例 6 10mg/ml FP3 融合蛋白在 4℃下 3ml 玻璃安瓿中的稳定性研究

[0079] FP3 融合蛋白 10mg/ml

[0080] 磷酸二氢钠 5mM

[0081] 海藻糖 10.0%

[0082] 精氨酸 100mM

[0083] PEG400 0.01%

[0084] pH 7.5 ～ 8.3

[0085] 将蛋白原液换液后，无菌分装得到 3ml 玻璃安瓿中，4℃留样，在 0，1，2，3，4，5，6，7，8，9，10，11，12 月测定样品，通过 SEC-HPLC 确定稳定性。

[0086] 表 6. 10mg/ml FP3 融合蛋白在 4℃的稳定性

[0087]

时间（月）	外观	pH 值	浓度（mg/ml）	聚合物（%）	亲和力（pM）
0	合格	8.3	10.4	0.3	10.1
1	合格	8.3	10.3	0.3	10.2
2	合格	8.2	10.2	0.5	10.0
3	合格	8.3	10.1	0.5	10.1
6	合格	8.1	10.2	0.9	9.9
9	合格	8.3	10.2	1.4	10.0
12	合格	8.2	10.1	4.2	9.8

[0088] 实施例 7 20mg/ml FP3 融合蛋白在 4℃下 3ml 玻璃安瓿中的稳定性研究

[0089] FP3 融合蛋白 20mg/ml

[0090] 柠檬酸 5mM

[0091] 蔗糖 4.0%

[0092] 氯化钠 4.0%

[0093] 精氨酸 100mM

[0094] 组氨酸 100mM

[0095] 吐温 20 0.05%

[0096] PEG400 0.05%

[0097] pH 7.5 ～ 8.3

[0098] 将蛋白原液换液后，无菌分装得到 3ml 玻璃安瓿中，4℃留样，在 0，1，2，3，4，5，6，7，8，9，10，11，12 月测定样品，通过 SEC-HPLC 确定稳定性。

[0099] 表 7. 20mg/ml FP3 融合蛋白在 4℃的稳定性

[0100]

时间（月）	外观	pH 值	浓度 (mg/ml)	聚合物（%）	亲和力 (pM)
0	合格	8.0	19.8	1	14.3
1	合格	7.8	20.7	1.5	10.2
2	合格	7.9	19.8	1.3	12.6
3	合格	7.9	20.6	2	10.5
4	合格	7.8	N/D	1.7	N/D
5	合格	7.8	N/D	2.2	N/D
6	合格	8.0	N/D	2.7	N/D

7	合格	7.8	N/D	2.7	N/D
8	合格	7.9	N/D	2.7	N/D
9	合格	7.9	19.5	2.7	12.5
10	合格	7.8	N/D	3.1	N/D
11	合格	7.8	N/D	3.5	N/D
12	合格	7.9	20.3	3.6	14.3

[0101] N/D 表示未检测。

[0102] 实施例 8 20mg/ml FP3 融合蛋白在 4℃下 3ml 玻璃安瓿中的稳定性研究

[0103] FP3 融合蛋白 20mg/ml

[0104] 柠檬酸 10mM

[0105] 蔗糖 30％

[0106] 精氨酸 500M

[0107] 吐温 20 0.1％

[0108] pH 7.5 ～ 8.3

[0109] 将蛋白原液换液后，无菌分装得到 3ml 玻璃安瓿中，4℃留样，在 0，1，2，3，4，5，6，7，8，9，10，11，12 月测定样品，通过 SEC-HPLC 确定稳定性。

[0110] 表 8. 20mg/ml FP3 融合蛋白在 4℃的稳定性

[0111]

时间（月）	外观	pH 值	浓度 (mg/ml)	聚合物（％）	亲和力 (pM)
0	合格	7.7	20.0	0.1	10.2
1	合格	7.9	20.0	0.1	10.2
2	合格	7.7	20.2	0.1	10.3
3	合格	7.8	20.2	0.2	10.3
6	合格	7.7	20.1	0.2	9.9
9	合格	7.9	20.3	0.3	10.0
12	合格	7.9	20.0	0.5	10.3

[0112] 实施例 9 10mg/ml FP3 融合蛋白在 4℃下 3ml 玻璃安瓿中的稳定性研究

[0113] FP3 融合蛋白 10mg/ml

[0114] 柠檬酸 10mM

[0115] 蔗糖 5％

[0116] 精氨酸 100M

[0117] 吐温 20 0.05%

[0118] pH 7.5 ～ 8.3

[0119] 将蛋白原液换液后，无菌分装得到 3ml 玻璃安瓿中，4℃留样，在 0，1，2，3，4，5，6，7，8，9，10，11，12 月测定样品，通过 SEC-HPLC 确定稳定性。

[0120] 表 9. 10mg/ml FP3 融合蛋白在 4℃的稳定性

[0121]

时间（月）	外观	pH 值	浓度 (mg/ml)	聚合物 (%)	亲和力 (pM)
0	合格	7.7	10.1	0.4	10.5
1	合格	7.9	10.1	0.5	10.4
2	合格	7.7	10.7	0.7	10.1
3	合格	7.8	10.6	0.8	10.0
6	合格	7.7	10.3	0.9	9.9
9	合格	7.9	10.1	1.8	7.7
12	合格	7.9	10.3	1.9	6.3

[0122] 实施例 10 10mg/ml FP3 融合蛋白在 4℃下 3ml 玻璃安瓿中的稳定性研究

[0123] 将 FP3 融合蛋白原液经 Vivaflow 浓缩换液后无菌分装后分装得到半成品，置 55mM 的柠檬酸，12.5% 的蔗糖，250mM 的精氨酸，0.05% 的吐温 20，pH7.9 缓冲液，调节 FP3 融合蛋白至 10mg/ml 的，无菌分装得到 3ml 玻璃安瓿中，4℃留样，在 0，1，2，3，4，5，6，7，8，9，10，11，12 月测定样品，通过 SEC-HPLC 确定稳定性。

[0124] 表 10. 10mg/ml FP3 融合蛋白在 4℃的稳定性

[0125]

时间（月）	外观	pH 值	浓度 (mg/ml)	聚合物 (%)	亲和力 (pM)
0	合格	8.3	10.0	0.2	9.7
1	合格	8.3	10.1	0.2	9.6
2	合格	8.2	10.2	0.3	9.6
3	合格	7.9	10.0	0.4	9.3
6	合格	8.0	10.1	0.5	9.3
9	合格	7.9	10.1	0.6	9.1
12	合格	7.9	10.3	1.0	9.4

[0126] 实施例 11 20mg/ml FP3 融合蛋白在 4℃下 3ml 玻璃安瓿中的稳定性研究

[0127] 将 FP3 融合蛋白原液经 Vivaflow 浓缩换液后无菌分装后分装得到半成品，置 55mM

的柠檬酸，12.5％的蔗糖，250mM 的精氨酸，0.05％的吐温 20，pH7.5 ～ 8.3 缓冲液，调节 FP3 融合蛋白至 20mg/ml 的，无菌分装得到 3ml 玻璃安瓿中，4℃留样，在 0，1，2，3，4，5，6，7，8，9，10，11，12 月测定样品，通过 SEC-HPLC 确定稳定性。

[0128] 表 11. 20mg/ml FP3 融合蛋白在 4℃的稳定性

[0129]

时间（月）	外观	pH 值	浓度（mg/ml）	聚合物（％）	亲和力（pM）
0	合格	7.9	19.9	0.7	10.7
1	合格	7.9	20.1	1.2	10.2
2	合格	7.7	20.7	1.3	10.3
3	合格	7.8	21.6	1.4	10.4
6	合格	8.0	21.6	1.4	10.4
9	合格	7.9	21.1	2.6	9.6
12	合格	7.9	21.3	3.6	9.4

[0130] 实施例 12 4℃下 40mg/ml FP3 融合蛋白冻干制剂的稳定性研究

[0131] FP3 融合蛋白 40mg/ml

[0132] 柠檬酸 250mM

[0133] 蔗糖 8.0％

[0134] 组氨酸 100mM

[0135] 吐温 20 0.10％

[0136] pH 7.5 ～ 8.3

[0137] 将 FP3 融合蛋白溶液调节到 40mg/ml，pH 调节到 7.5 ～ 8.3 后分装后分装到 3ml 玻璃安瓿中，优化的冻干曲线的条件为：-50℃迅速预冻 4 个小时，控制在 -20℃升华除去大部分水分，然后逐步升高隔板的温度进一步去除残留的水分，最后一步应用 25 度并达到极限真空，使水分降低，同时使样品温度升得不会太高。冻干结束后，亚塞，出冻干箱，轧铝盖，进行水分和纯度等分析，4℃留样，在 0，1，6，12 月加入等体积注射用水复溶，测定样品，通过 SEC-HPLC 确定稳定性。

[0138] 表 12. 40mg/ml FP3 融合蛋白在 4℃的稳定性

[0139]

时间（月）	复溶后外观	pH 值	浓度（mg/ml）	聚合物（％）	亲和力（pM）
0	合格	7.9	39.9	0.1	11.2
1	合格	7.9	40.1	0.2	10.6
6	合格	7.9	40.7	0.3	10.1
12	合格	8.3	40.6	0.4	10.0

[0140] 实施例 13 4℃下 40mg/ml FP3 融合蛋白冻干制剂的稳定性研究

[0141] FP3 融合蛋白 40mg/ml

[0142] 柠檬酸 10mM

[0143] 蔗糖 5%

[0144] 精氨酸 100mM

[0145] 吐温 20 0.05%

[0146] pH 7.5 ～ 8.3

[0147] 将 FP3 融合蛋白溶液调节到 20mg/ml，pH 调节到 7.5 ～ 8.3 后分装后分装到 3ml 玻璃安瓿中，优化的冻干曲线的条件为 :-50℃迅速预冻 4 个小时，控制在 -20℃升华除去大部分水分，然后逐步升高隔板的温度进一步去除残留的水分，最后一步应用 25 度并达到极限真空，使水分降低，同时使样品温度升得不会太高。冻干结束后，亚塞，出冻干箱，轧铝盖，进行水分和纯度等分析，4℃留样，在 0，1，6，12 月加入等体积注射用水复溶，测定样品，通过 SEC-HPLC 确定稳定性。

[0148] 表 13. 40mg/ml FP3 融合蛋白在 4℃的稳定性

[0149]

时间（月）	复溶后外观	pH 值	浓度 (mg/ml)	聚合物（%）	亲和力 (pM)
0	合格	8.0	40	0.1	10.2
1	合格	8.3	40	0.1	10.5
6	合格	7.9	40	0.2	10.2
12	合格	8.2	40.1	0.3	10.3

[0150] 实施例 14 4℃下 20mg/ml FP3 融合蛋白在玻璃预填充注射器中的稳定性研究

[0151] FP3 融合蛋白 20mg/ml

[0152] 柠檬酸 55mM

[0153] 蔗糖 12.5%

[0154] 精氨酸 250mM

[0155] 吐温 20 0.05%

[0156] pH 7.5 ～ 8.3

[0157] 将 FP3 融合蛋白原液经浓缩换液后无菌分装后分装得到半成品，调节 FP3 融合蛋白至 20mg/ml 的，无菌分装到具有 FluroTec 涂层的 1ml 玻璃玻璃预填充注射器中，4℃留样，在 0，1，6，12 月测定样品，通过 SEC-HPLC 确定稳定性。

[0158] 表 14. 4℃下 20mg/ml FP3 融合蛋白在玻璃预填充注射器中的稳定性研究

[0159]

时间（月）	外观	pH 值	浓度 (mg/ml)	聚合物（%）	亲和力 (pM)
0	合格	7.9	20.0	0.1	11.2
1	合格	7.9	20.1	0.1	11.6
6	合格	7.9	20.1	0.1	11.1
12	合格	8.2	20.1	0.1	11.0

[0160] 实施例 15 聚合物的凝胶排阻色谱分析 (SEC-HPLC)

[0161] 多孔硅胶柱稳定性高、耐热耐压、寿命长、能实现生物大分子的快速分离。TSK G3000SWxl 为多孔硅胶柱，粒径 5μm、孔径 250 埃、分离球蛋白的范围 10-500kD，分离样品时间 30min，是国际上分析生物大分子普遍使用的色谱柱。本发明中，我们选用多孔硅胶柱 TSKG3000SWxl 色谱柱，仪器为 Waters 的 2695 高效液相色谱仪，以 pH 至 7.20 的磷酸盐缓冲液为流动相，流速为 0.5ml/ml，柱温为 25℃，检测波长为 280nm，对重组人血管内皮生长因子受体－抗体 FP3 融合蛋白的纯度进行检测。

[0162] 实施例 16 兔玻璃体注射重复给药试验

[0163] 12 只日本大耳白兔，随机分为 2 组，分别是供试品组和溶剂对照组，每组 6 只，雌雄各半。在各组动物的右眼，分别单次玻璃体注射给予供试品 (0.5mg/50μL/ 眼）或等体积溶剂对照品，另一侧做假注射对照或空白对照。给药后每日进行肉眼观察，定期使用间接检眼镜及裂隙灯进行检查。给药后 14 天处死动物，取眼球进行组织病理学检查。通过观察结果及组织病理学检查结果，考察供试品的局部毒性。结果发现：日本大耳白兔眼玻璃体内注射给药，6 只给予供试品的眼睛中，有 1 只肉眼观察见有轻微充血，1 只见少量分泌物；裂隙灯观察见 1 只出现晶状体混浊。同时，分别在给予溶剂对照侧、假注射侧及空白对照侧眼睛观察到相同现象，且发生率及发生时间与给予供试品侧眼睛基本一致。由此可排除这些现象是由供试品引起的刺激反应。组织病理学检查见所有眼球组织结构均正常。故在本次试验条件下，兔眼单次玻璃体注射给予重组人血管内皮生长因子受体－抗体融合蛋白注射液，未引起局部刺激性或组织损伤反应。

[0001]

序 列 表

[0002]

<110> 成都康弘生物科技有限公司

<120> 一种含有抑制血管增生的融合蛋白的药物组合物及用途

<130> ZL200610066257.2

<160> 1

<170> PatentIn version 3.3

<210> 1

<211> 552

<212> PRT

<213> 人工序列

<400> 1

```
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
1               5                  10                  15
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu
            20                  25                  30
Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu
        35                  40                  45
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu
    50                  55                  60
Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
65                  70                  75                  80
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu
                85                  90                  95
Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys
            100                 105                 110
Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val
        115                 120                 125
Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val
    130                 135                 140
Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn
145                 150                 155                 160
Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg
                165                 170                 175
Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr
            180                 185                 190
Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys
        195                 200                 205
Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg
```

[0003]

210 215 220

Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu
225 230 235 240

Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Ala Lys Tyr Leu
245 250 255

Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Leu
260 265 270

Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr Ile Met Glu
275 280 285

Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro
290 295 300

Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val Val Tyr Val
305 310 315 320

Pro Pro Gly Pro Gly Asp Lys Thr His Thr Cys Pro Leu Cys Pro Ala
325 330 335

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
340 345 350

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
355 360 365

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
370 375 380

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
385 390 395 400

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
405 410 415

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
420 425 430

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
435 440 445

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
450 455 460

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
465 470 475 480

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
485 490 495

Lys Ala Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
500 505 510

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
515 520 525

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
530 535 540

Ser Leu Ser Leu Ser Pro Gly Lys
545 550

Notice

ABSTRACT CN103212075B

[0001]

13 The present invention relates to an eye drop containing a VEGF antagonist. The eye drop contains a low concentration of VEGF antagonist, especially containing 0.05-9.0 mg/ml VEGF antagonist. The eye drops of the present invention ensure safety and stability. Its curative effect is remarkable on the basis of the.

CN103212075B Eye drop containing VEGF antagonist

Technical field

The invention relates to the field of pharmaceutical preparations, in particular to an eye drop containing a VEGF antagonist.

Background technique

The incidence of ocular surface diseases such as corneal neovascularization, neovascular glaucoma, pterygium, and chronic conjunctivitis is related to the production of new blood vessels. Overexpression of VEGF can induce ocular surface angiogenesis. The corneal neovascularization is not an independent corneal disease, but a pathological change. There are many causes of corneal neovascularization, immunoinflammatory, infectious, degenerative, traumatic, improper use of contact lenses, iatrogenic diseases, and the like. If the corneal neovascularization caused by the above reasons is not treated in time, it will eventually lead to corneal blindness. Corneal neovascularization is the leading cause of vision loss and blindness worldwide. About 4.14% of ophthalmic patients in the United States have corneal neovascularization. In the United States, there are 1.4 million new corneal neovascularization patients each year, 12% of which cause vision loss. There are approximately 170,000 new corneal neovascularization patients with decreased vision each year in the United States. There is no epidemiological investigation of corneal neovascularization in China. If the corneal neovascularization accounts for 4% of ophthalmic patients, 12% of them will cause visual loss. In 2009, the national ophthalmology patients were 62.83 million, and there were about 300,000 in the country each year. New patients have affected vision due to corneal renewal. The progression of corneal neovascularization leads to a further deterioration of vision, which ultimately requires a corneal transplant to restore vision. Therefore, such patients need effective anti-angiogenic therapy to prevent further loss of vision, while avoiding corneal transplantation, and doctors have no effective treatment for corneal neovascularization.

Many properties of biopharmaceuticals, especially recombinant proteins, are completely different from small molecule chemical molecules, and their unstable degradation reaction is a multi-step reaction. Limited to the limitations of current analytical methods, these multi-step degradations that occur in advanced structures are also difficult to measure accurately, and their stability, especially under normal conditions, is a significant challenge. The eye is one of the most important organs of the human body: nearly 80% of the brain's information comes from the eye; at the same time, the eye is a relatively weak tissue due to its special physiological structure, and its requirements for viscosity and osmotic pressure are very high. Therefore, the ophthalmic preparations used in ophthalmic preparations, especially in the case of trauma, are extremely strict.

The biological effects of VEGF (vascular endothelial growth factor) are mediated through its specific receptor VEGFR (vascular endothelial growth factor receptor), which leads to ligand-mediated dimerization. Dimerization of the receptor promotes autophosphorylation and dephosphorylation of adjacent receptor subunits, triggering signal transduction. VEGFR is a tyrosine protein kinase, which is classified into its function and structure: fins-like tyrosine kinase-1 (VEGFR-1/Flt-1) and kinase insertion region receptor (VEGFR-2/Flk-1/ KDR), fins-like tyrosine kinase-4 (VEGFR-3/Flt-4) and some low relative molecular mass VEGFR (neuropilin-1). Flt-1 and KDR are mainly distributed on vascular endothelial cells, while Flt-4 is mainly distributed on lymphatic endothelial cells. The main binding to VEGF is Flt-1 and KDR. Flt-1 binds to VEGF more strongly than KDR. They are all glycosylated transmembrane receptors and directly participate in the signal transduction of VEGF into cells. The combination of Flt-1 and VEGF can promote the formation of vascular endothelial cells and regulate vascular permeability; KDR combined with VEGF can promote the proliferation and maturation of vascular endothelial cells. VEGF antagonists, especially a class of engineered targeted genetically engineered proteins, such as Lucentis, Avastin, VEGF-trap, etc., which effectively block vascular endothelial growth factor (VEGF)-mediated signaling and inhibit pathology The growth of new blood vessels is used to treat diseases caused by neovascularization such as tumors and eyes, but the marketed preparation form of the above-mentioned drugs is an injection, which is administered by direct injection or systemic administration of a vitreous body in the form of a preparation for a patient. In other words, its

compliance is poor and there are many inconveniences. Therefore, the researchers began to study a patient-friendly, convenient and effective form of a pharmaceutical preparation containing a VEGF antagonist, and US Pat. No. 7,303,748 discloses an eye drop containing VEGF-trap, the formulation of which is 39.4-103.06 mg/ml. VEGF-trap, 5 mM phosphoric acid, 5 mM citric acid, 100 mM sodium chloride, 0.005% Tween-20. However, the concentration of the eye drops is relatively large, and since the biological preparations generally have instability problems, many patients with anti-angiogenic treatments on the ocular surface are currently reluctant to be hospitalized, and high-concentration biological preparations are liable to cause inconvenience in storage and use, and at the same time Ocular surface administration is a special route of administration. For pH, osmotic pressure and local irritation, bacteriostatic agents and antibacterial agents cannot be added. The above problems make the use of anti-angiogenic drugs for ocular surface treatment expensive and limited. . Therefore, it provides an eye drop with a low concentration and a curative effect on inhibiting neovascularization, and is used for treating various ocular surface diseases such as corneal neovascularization, neovascular glaucoma, pterygium, chronic conjunctivitis, etc. Demand has a significant meaning.

Summary of the invention

One of the technical problems to be solved by the present invention is to provide an eye drop containing a VEGF antagonist which is small in concentration but effective in curative effect.

In order to solve the above technical problem, the present invention provides the following technical solutions:

In one aspect, the present invention provides an eye drop solution containing a VEGF antagonist, which comprises 0.05-9.0 mg/ml of a VEGF antagonist; preferably contains 0.1-1.0 mg/ml of a VEGF antagonist; more preferably 0.5 mg/ml of a VEGF antagonist; wherein the VEGF antagonist is preferably a fusion protein comprising a fragment of FLT-1 and KDR.

The above VEGF antagonist preferably has a fusion protein of one of the following structures:

a. FP1, a protein obtained by fusing a second immunoglobulin-like region of FLT-1 and a third immunoglobulin-like region of KDR with a human immunoglobulin Fc fragment: FLTd2-KDRd3-Fc;

b. FP2, a protein obtained by fusion of a first immunoglobulin-like region of KDR, a second immunoglobulin-like region of FLT-1, and a third immunoglobulin-like region of KDR with a human immunoglobulin Fc fragment:

KDRd1-FLTd2-KDRd3-Fc;

c. FP3, a protein obtained by fusing a second immunoglobulin-like region of FLT-1 and a 3-4 immunoglobulin-like region of KDR with a human immunoglobulin Fc fragment: FLTd2-KDRd3,4-Fc;

d. FP4, a protein obtained by fusing a second immunoglobulin-like region of FLT-1, a third immunoglobulin-like region of KDR, and a fourth immunoglobulin-like region of FLT-1 and a human immunoglobulin Fc fragment. :

FLTd2-KDRd3-FLTd4-Fc;

e.FP5, a protein obtained by fusing a second immunoglobulin-like region of FLT-1 and a 3-5 immunoglobulin-like region of KDR with a human immunoglobulin Fc fragment: FLTd2-KDRd3, 4, 5-Fc ;or

f.FP6, which is composed of a second immunoglobulin-like region of FLT-1, a third immunoglobulin-like region of KDR, and a 4-5 immunoglobulin-like region of FLT-1 fused with a human immunoglobulin Fc fragment. Protein:

FLTd2-KDRd3-FLTd4, 5-Fc;

g.FP7, a protein obtained by fusing a second immunoglobulin-like region of FLT-1 and a 3-4 immunoglobulin-like region of KDR: FLTd2-KDRd3, 4;

h.FP8, a protein obtained by fusing a second immunoglobulin-like region of FLT-1 and a third immunoglobulin-like region of KDR: FLTd2-KDRd3.

The amino acids FLT-1D2, FLT-1D4, KDRD1, KDRD3, and KDRD4 of the above FLT-1 and KDR immunoglobulin-like regions are shown in Table 1-5. The amino acid sequence of FP3 protein is shown in Table 6. The amino acid sequence of FP1 protein is listed. Listing 7, the amino acid sequence of the FP7 protein is shown in Sequence Listing 8, and the amino acid sequence of the FP8 protein is shown in Sequence Listing 9.

The eye drops provided by the present invention may further contain one or more of the following components:

(a) 5-100 mM buffer, wherein the acid is selected from the group consisting of Tris-HCl, citric acid, phosphoric acid, sodium hydrogen phosphate, sodium dihydrogen phosphate, acetic acid, succinic acid, hydrochloric acid;

(b) a 5-500 mM basic amino acid selected from the group consisting of lysine, arginine, and histidine, or a combination thereof;

(c) 0.1-30% salt osmotic pressure regulator, wherein the sugar is selected from one or more of sucrose, trehalose, mannitol, glycerin, propylene glycol, sorbitol, the salt is selected from sodium chloride or other One or a combination of pharmaceutically acceptable salts;

(d) 0.005-0.1% of one or more surfactants or cosolvents selected from the group consisting of polyethylene glycol, Tween 20, Tween 80, propylene glycol, dimethyl sulfoxide or other pharmaceutically acceptable surface One or more of the active agents;

The solution of one or more of the above components is formulated to have a pH of from 6 to 8.3.

The VEGF antagonist described in the above eye drops is most preferably a fusion protein of SEQ ID No: 6.

The present invention still further provides an eye drop containing the following components, the specific components are as follows:

(a) a fusion protein of SEQ ID No: 6 of 0.05-9 mg/ml;

(b) 5-250 mM citric acid;

(c) one or two of 5-500 mM arginine or histidine;

(d) 4-30% sucrose or trehalose;

(e) 0.01 to 0.1% of a surfactant or cosolvent selected from one or both of polyethylene glycol or Tween 20;

(f) The pH is adjusted to 7.5 to 8.3.

The present invention still further provides an eye drop containing the following components, the specific components are as follows:

(a) 0.1-1 mg/ml of the fusion protein of SEQ ID No: 6;

(b) 10-50 mM citric acid;

(c) one or both of 50-100 mM arginine or histidine;

(d) 5-20% sucrose;

(e) 0.01 to 0.1% of a surfactant or cosolvent selected from one or both of polyethylene glycol or Tween 20;

(f) The pH is adjusted to 7.5 to 8.3.

The present invention further provides an eye drop containing the following components, the specific components are as follows:

(a) 0.5 mg/ml of the fusion protein of SEQ ID No: 6;

(b) 50 mM citric acid;

(c) 250 mM arginine;

(d) 12.5% sucrose;

(e) 0.05% Tween-20;

(f) The pH is adjusted to 7.5 to 8.3.

The present invention also provides the use of the above eye drops in the preparation of a medicament for treating ocular surface diseases caused by angiogenesis or growth; preferably, the ocular surface disease is neovascularization, corneal neovascularization, ocular surface neovascularization after corneal transplantation or Any of the pterygium or its complications.

The technical contents disclosed in the Chinese Patent No. ZL200510073595.4 and ZL200610066257.2 are incorporated by reference in the present application. The technical contents of the Chinese unpublished patent CN 201010267503.7 are also incorporated herein by reference.

Compared with the prior art, the invention has the advantages that the content of the fusion protein in the eye drops is extremely low and the curative effect is exact, and the production cost can be greatly saved on the basis of the high stability of the low concentration eye drops.

Detailed ways

The following examples are merely illustrative of the invention and are not to be construed as limiting the scope of the invention.

Example 1. Study on Corneal Neovascularization Caused by Alkali Burn by Compaqip Eye Drops

Drug: chlortetracycline eye ointment, specification batch number: 2.0g / support, 411002, valid until 2014.12, Chongqing Kerui Pharmaceutical Co., Ltd.; lidocaine hydrochloride injection, specification batch number: 5ml / support, 0.1g / support, Valid until 2012.04., Tianjin Pharmaceutical Jiaozuo Co., Ltd.

Reagents: sodium hydroxide (NaOH), specification batch number: 500g / bottle, 20091223, Chengdu Kelon Chemical Reagent Factory.

Test sample:

A, Compaqip eye drops (prepared according to Example 3) 10mg / ml, colorless transparent liquid, 1ml / support, batch number: FR1108001, stored at 2 ~ 8 ° C, dripping into the surface of the eye when used;

B, Compaqip eye drops (prepared according to Example 3) 9mg / ml, colorless transparent liquid, 1ml / support, batch number: FR1108002, stored at 2 ~ 8 ° C, dripping into the surface of the eye when used;

C, dexamethasone, colorless transparent liquid, 1ml / support, batch number: FR1108003, stored at 2 ~ 8 ° C, dripping into the surface of the eye when used;

D, Compaqip eye drops (prepared according to Example 3) 0.1 mg / ml, colorless transparent liquid, 1 ml / support, batch number: FR1108004, stored at 2 ~ 8 ° C, dripping into the surface of the eye when used;

E, without Compaq preparation buffer (prepared according to Example 16), colorless transparent liquid, 1 ml / support, batch number: FR1108005, stored at 2 ~ 8 ° C, added to the surface of the eye when used;

F, Compaqip eye drops (prepared according to Example 3) 5mg / ml, colorless transparent liquid, 1ml / support, batch number: FR1108006, stored at 2 ~ 8 ° C, dripping into the surface of the eye when used;

They are all provided by the preparation room of Sinochem Pharmaceutical Research Department of Kanghong Pharmaceutical Group Enterprise Technology Center.

Experimental methods and results:

Take 48 healthy New Zealand rabbits, call their body weight, anesthetize with 3% pentobarbital sodium (1ml/kg), and apply local anesthetic lidocaine hydrochloride to the surface of the eyes at a dose of 20μl/eye; preparation 9mm diameter filter paper, immersed in 1mol / L NaOH solution for about 10s, put the filter paper on the dry filter paper with tweezers to absorb excess NaOH solution, and put the filter paper soaked with NaOH in the middle of the rabbit cornea for 60s Remove, quickly take the bottle and rinse the cornea with about 20ml of normal saline, and give antibiotics to prevent infection (chlortetracycline hydrochloride eye ointment), 3 times / 2 days.

Immediately after modeling, it was divided into burn, A (Compaqic eye drops (prepared according to Example 3) 10 mg/ml), B (Compaqic eye drops (prepared according to Example 3) 9 mg/ml), C (dexamethasone), D (Compaqicip eye drops (prepared according to Example 3) 0.1 mg/ml), E (Compaq-free formulation buffer solution (prepared according to Example 16)) and F (Compaqip eye drops (prepared according to Example 3) 5 mg / ml) seven groups, the day of modeling is 0 days, from the first day, A ~ F group given the corresponding drug, the frequency of administration 6 times / Day, the dose was 50 μl/eye each time, continuous administration for 10 days, and another 2 New Zealand rabbits were set as the normal group. At the same time as daily administration, the corneal NV growth state and the ocular inflammatory reaction were observed.

On the 10th day after administration, the animals were anesthetized with 3% sodium pentobarbital solution (1 ml/kg), and local anesthetic lidocaine hydrochloride solution was applied to the surface of the eyes at a dose of 20 μl/eye, 10 times the objective lens in the slit lamp. The cornea NV clock direction was observed under the rabbit eye while photographing under a 10x and 16x objective lens. The images were acquired in Photoshop CS for clock point correction, and the corneal area was treated with Image Pro Plus; the area formula: $S=C/12\times3.1416\times[R2-(RL)2]$, where C indicates that the corneal edge from the NV to the picture The number of hours occupied by the point when no NV is grown, R is the length from the edge of the contact between the cornea and the sclera in the picture to the center of the cornea, and L is the NV from the root of the contact point of the cornea and sclera in the picture to the end of the NV in the cornea. Length, the longest one of the blood vessels in each hour. All statistical analysis of the data was performed by T test analysis of variance. The data is expressed as

and is the average and s is the standard deviation.

According to the general ocular surface observation, the vascular network of the cornea at the edge of burns in the burned eyes was obvious on the 1st and 2nd day of burns. On the 3rd day of burn, there was NV growth at the corneal edge; on the 5th day, Compaqip eye drops 5mg/ml and no NV growth on the corneal surface of the buffer solution containing Compaqcept was obvious, dexamethasone, Compaqip eye drops 10 mg/ml, Compaqip eye drops 9 mg/ml and Compaqip eye drops 0.1 The growth of NV in the mg/ml group was not obvious; on the 7th day, the NV growth of the cornea surface was obvious in the eyes of the group, and the corneal NV of the burn group and the buffer solution containing no compaqing group grew to the burned cornea, and some NV had grown to the edge of the burned cornea. , dexamethasone, Compaqip eye drops 10mg/ml, Compaqip eye drops 9mg/ml, Compaqip eye drops 5mg/ml and Compaqip eye drops 0.1mg/ml There was very little NV growth in the group to the edge of the burned cornea; on the 10th day, the burn group and the buffer solution solution without Compaqcept had NV into the burned cornea, while dexamethasone and Compaqip 10 mg/ml, Compaqip eye drops 9mg/ml, Compaqip 5mg/ml and Compaqip 0.1mg/ml group NV had less burned cornea.

Corneal neovascularization area effect: On the 10th day, the corneal NV area data analysis results showed that dexamethasone, Compaqip 10mg/ml, Compaq compared with the alkali burn group and the compatibilizer-free preparation buffer solution group. Xipu eye drops 9mg/ml, Compaqip 5mg/ml and Compaqip 0.1mg/ml can significantly inhibit corneal NV growth and reduce NV area, which is statistically significant (P(0.05), of which dexamethasone It was the best to inhibit NV growth with Compaqep 0.1 mg/ml (P(0.01). Although the formulation buffer solution without Compaqcept did not inhibit corneal neovascularization and the NV area was larger than that of the burn group, there was no significant difference. The results are shown in Table 1.

Table 1 Effect of various drugs on corneal corneal NV caused by alkali burn

Note: The negative control is a drug-free buffer, and the positive control is dexamethasone. Compared with the burn group, *P(0.05, **P(0.01; compared with the B group, #P(0.05,##P(0.01 Compared with the E group, ^P(0.05, ^^P(0.01.

Example 2 Effect of low-dose Compaqip eye drops on corneal NV growth induced by alkali burn

Drug: chlortetracycline eye ointment, specification batch number: 2.0g / support, 411002, valid until 2014.12, Chongqing Kerui Pharmaceutical Co., Ltd.; lidocaine hydrochloride injection, specification batch number: 5ml / support, 0.1g / support, Valid until 2012.04., Tianjin Pharmaceutical Jiaozuo Co., Ltd.

Reagents: sodium hydroxide (NaOH), specification batch number: 500g / bottle, 20091223, Chengdu Kelon Chemical Reagent Factory.

Test sample:

A, Compaqip eye drops (prepared according to Example 3) 0.5 mg / ml, colorless transparent liquid, 800 μl / support, batch number: 20111001, stored at 2 ~ 8 ° C, used to drop into the surface of the eye;

B, Compaqip eye drops (prepared according to Example 3) 0.1 mg / ml, colorless transparent liquid, 800 μl / support, batch number: 20111001, stored at 2 ~ 8 ° C, used to drop into the surface of the eye;

C, without Compaqep formulation buffer solution, colorless transparent liquid, 800μl / support, batch number: 20111001, stored at 2 ~ 8 ° C, dripping into the surface of the eye when used;

D, Compaqip eye drops (prepared according to Example 3) 0.01 mg / ml, colorless transparent liquid, 800 μl / support, batch number: 20111001, stored at 2 ~ 8 ° C, used to drop into the surface of the eye;

E, dexamethasone, colorless transparent liquid, 800μl / support, batch number: 20111001, stored at 2 ~ 8 ° C, dripping into the surface of the eye when used;

F, Compaqip eye drops (prepared according to Example 3) 0.05 mg / ml, colorless transparent liquid, 800 μl / support, batch number: 20111001, stored at 2 ~ 8 ° C, dripping into the surface of the eye when used;

G, Compaqip eye drops (prepared according to Example 3) 1 mg / ml, colorless transparent liquid, 800 μl / support, batch number: 20111001, stored at 2 ~ 8 ° C, added to the surface of the eye when used;

They are all provided by the preparation room of Sinochem Pharmaceutical Research Department of Kanghong Pharmaceutical Group Enterprise Technology Center.

Experimental methods and results:

The test was divided into A (Compaqip eye drops (prepared according to Example 3) 0.5 mg/ml), B (Compaqip eye drops (prepared according to Example 3) 0.1 mg/ml), C (not Formulation buffer containing Compaqip (prepared according to Example 16), D (Compaqip eye drops (prepared according to Example 3) 0.01 mg/ml), E (dexamethasone), F (Compaqip eye drops (prepared as in Example 3) 0.05 mg/ml) and G (Compaqip eye drops (prepared according to Example 3) 1 mg/ml) were in eight groups.

The test method was operated in the same manner as in Example 1.

According to ocular surface observation, the vascular network of the cornea at the edge of burns in the burned eyes was obvious, and some eyelids were red and swollen on the first day and the second day of burn. On the third day of burn, there was NV growth trend at the corneal edge, and the eyelid redness and swelling disappeared. On the fifth day, each group There were NV growth on the corneal surface, but some rabbit eyelids appeared red and swollen. On the 7th day, the NV growth of the cornea surface was obvious, and there was obvious centripetal growth, while the dexamethasone group had less corneal NV, but the rabbit eyelid swelling

and swelling phenomenon. On the 10th day, the corneal NV of each group containing Compaqip was obviously grown, and some corneal neovascularization had entered the burn site. In the dexamethasone group, NV grew into the corneal burn site less, and the rabbit eyelid redness and swelling did not appear. Recovery, and the group containing Composib did not have redness and swelling of the eyelids of the rabbit.

Corneal neovascularization area effect: On the 10th day, the corneal NV area data analysis results showed that dexamethasone and Compaqip eye drops 0.5 mg/ml were significantly inhibited compared with the compatibilizer-free preparation buffer solution group. Corneal NV growth, reducing NV area, was statistically significant (P (0.05). Compaqip eye drops 1 mg/ml and Compaqip eye drops 0.1 mg/ml also inhibited corneal NV growth. Compaqip eye drops 0.01mg/ml did not inhibit corneal neovascularization, and the NV area was larger than the burn group, but there was no significant difference. See Table 2.

Table 2 Effect of various drugs on corneal corneal NV caused by alkali burn

Note: Buffer control, *P(005, **P(001.

Example 3 Preparation of Compaqep (FP3 Fusion Protein) Eye Drops

prescription:

FP3 fusion protein 10mg/ml, 9mg/ml, 5mg/ml, 1mg/ml, 0.5mg/ml, 0.1mg/ml, 0.05mg/ml or 0.01mg/ml

Preparation method: After concentrating and defrosting the FP3 fusion protein after concentration, the solution is added to the filter-sterilized 55 mM citric acid, 12.5% sucrose, 250 mM in the C-class cleansing and cleaning station (sterile cabinet) according to the aseptic method. Arginine and 0.05% Tween 20 buffer, filtered, and adjusted FP3 fusion protein to 10mg/ml, 9mg/ml, 5mg/ml, 1mg/ml, 0.5mg/ml, 0.1mg/ml, 0.05mg /ml or 0.01mg/ml, pH 7.5 ~ 8.3, aseptically dispensed into a container containing eye drops, stored at 2-8 ° C.

Example 4 Preparation of Composip (FP3 Fusion Protein) Eye Drops

Prescription:

Preparation method: After concentrating and defrosting the FP3 fusion protein after concentration, the solution is added to the filter-sterilized 10 mM citric acid, 8.0% sucrose, 5 mM in the C-class cleansing and cleaning station (sterile cabinet) according to the aseptic method. Arginine and 0.05% Tween 20 buffer, filter, adjust FP3 fusion protein to 0.5mg/ml, pH 7.5 ~ 8.3, aseptically dispense into the container containing the eye drops, stored in 2-8 °C.

Example 5 Preparation of Composip (FP3 Fusion Protein) Eye Drops

prescription:

Preparation method: After concentrating and defrosting the FP3 fusion protein after concentration, the solution is added to the filter-sterilized 100 mM citric acid, 20.0% sucrose, 250 mM in a C-class cleansing and cleaning station (sterile cabinet). Arginine and 0.10% Tween 20 buffer, filtered, adjusted FP3 fusion protein to 1.0mg/ml, pH 7.5 ~ 8.3 aseptically packed into a container containing eye drops, stored at 2-8 ° C .

Example 6 Preparation of Composip (FP3 Fusion Protein) Eye Drops

prescription:

The preparation method comprises the following steps: defrosting the FP3 fusion protein after concentration and changing, and adding 5 mM sodium dihydrogen phosphate and 10.0% seaweed by filtration in a C-class cleansing and cleaning station (sterile cabinet) according to an aseptic method. Sugar, 100 mM arginine and 0.01% PEG400 buffer, filtered, adjusted FP3 fusion protein to 0.1 mg / ml, pH 7.5 ~ 8.3, aseptically dispensed into a container containing eye drops, stored in 2- 8 ° C.

Example 7 Preparation of Composip (FP3 Fusion Protein) Eye Drops

prescription:

Preparation method: After concentrating and thawing the FP3 fusion protein after concentration and changing, in the C-class cleansing and cleaning station (sterile cabinet), the aseptic method is added to filter and sterilize containing 5 mM citric acid, 4.0% sucrose, 4.0. % sodium chloride, 100 mM arginine, 100 mM histidine, 0.05% Tween 20, 0.05% PEG400 buffer, filtered, adjusted FP3 fusion protein to 0.05 mg/ml, pH 7.5-8.3, sterile Store in a container containing eye drops and store at 2-8 °C.

Example 8 Preparation of Composip (FP3 Fusion Protein) Eye Drops

prescription:

Preparation method: After concentrating and defrosting the FP3 fusion protein after concentration, the solution is added to the filter-sterilized 10 mM citric acid, 30.0% sucrose, 500 mM in the C-class cleansing and cleaning station (sterile cabinet) according to the aseptic method. Arginine, 0.1% Tween 20 buffer, filtered, adjusted FP3 fusion protein to 5.0mg/ml, pH 7.5 ~ 8.3, aseptically packed into a container containing eye drops, stored in 2-8 °C.

Example 9 Preparation of Composip (FP3 Fusion Protein) Eye Drops

prescription:

Preparation method: After concentrating and defrosting the FP3 fusion protein after concentration, the solution is added to the filter-sterilized 10 mM citric acid, 5.0% sucrose, 100 mM in the C-class cleansing and cleaning station (sterile cabinet) according to the aseptic method. Arginine, 0.05% Tween 20 buffer, filtered, adjusted FP3 fusion protein to 0.5mg/ml, pH 7.5 ~ 8.3, aseptically packed into a container containing eye drops, stored in 2-8 °C.

Example 10 Preparation of Composip (FP3 Fusion Protein) Eye Drops

prescription:

Preparation method: After concentrating and defrosting the FP3 fusion protein after concentration, the solution is added to the filter-sterilized method containing 250 mM citric acid, 8.0% sucrose, 100 mM in the C-stage cleansing and cleaning station (sterile cabinet). Histidine, 0.10% Tween 20 buffer, filtered, adjusted FP3 fusion protein to 0.5mg/ml, pH 7.5 ~ 8.3, aseptically packed into a container containing eye drops, stored in 2-8 °C.

Example 13 Preparation of Composip (FP3 Fusion Protein) Eye Drops

prescription:

Preparation method: After concentrating and defrosting the FP3 fusion protein after concentration, the solution is added to the filter-sterilized 10 mM citric acid, 5.0% sucrose, 100 mM in the C-class cleansing and cleaning station (sterile cabinet) according to the aseptic method. Arginine, 0.05% Tween 20 buffer, filtered, adjusted FP3 fusion protein to 0.5mg/ml, pH 7.5 ~ 8.3, aseptically packed into a container containing eye drops, stored in 2-8 °C.

Preparation of Example 14 Series Fusion Protein Eye Drops

prescription:

Preparation method: After concentrating and thawing various fusion proteins (FP1, FP2, FP4, FP5, FP6, FP7, FP8) after concentration and liquidation, in the C-class cleansing and cleaning station (aseptic cabinet), aseptic operation The filter was added to a filter containing 55 mM citric acid, 12.5% sucrose, 250 mM arginine and 0.05% Tween 20, and filtered to adjust the fusion protein to 9 mg/ml, 5 mg/ml, 1 mg/ml, 0.5 mg, respectively. /ml, 0.1mg/ml or 0.05mg/ml, pH 6.0-8.3, aseptically dispensed into a container containing eye drops and stored at 2-8 °C.

Example 15 Preparation of FP1 Fusion Protein Eye Drops

prescription:

Preparation method: After concentrating and defrosting the FP1 fusion protein after concentration, the solution is added to the filter-sterilized 5 mM phosphoric acid, 5 mM citric acid, 100 mM chlorine in a C-class clean-cleaning station (sterile cabinet) according to the aseptic method. Sodium hydride and 0.005% Tween 20 buffer, filtered, adjusted fusion protein to 1.0 mg/ml, pH 6.0-8.0, aseptically dispensed into a container containing eye drops, stored at 2-8 °C.

Example 16 Preparation of Formulation Buffer Without Compaqep (FP3 Fusion Protein)

prescription:

Preparation method: the aseptic method is added to the filter-sterilized buffer containing 55 mM citric acid, 12.5% sucrose, 250 mM arginine and 0.05% Tween 20, filtered, and the pH is adjusted to 7.5-8.3, respectively. Dispense into a container containing eye drops and store at 2-8 °C.

Example 17 Stability of 0.1 mg/ml Composip (FP3 fusion protein) eye drops at 25 ° C

1、Prepare 0.1 mg/ml Compaqip (FP3 fusion protein) eye drops according to Example 3.

2、The above 0.1 mg/ml Compaqip (FP3 fusion protein) eye drops were taken at 25 ° C, at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 The samples were assayed monthly and stability was determined by SEC-HPLC. The results show that the prescription can effectively inhibit the formation of the polymer, the purity of the product does not decrease substantially, and the affinity of the fusion protein to VEGF is almost unchanged.

The specific results are shown in Table 3.

Table 3. Stability of 0.1 mg/ml FP3 fusion protein at 25 ° C

Time (month) Appearance pH Polymer (%) Affinity (pM) 0 Qualified 7.2 0.01 10.6 1 Qualified 7.4 0.03 10.5 2 Qualified 7.5 0.03 10.4 3 Qualified 7.5 0.05 10.4 6 Qualified 7.7 0.07 10.2 9 Qualified 7.6 0.08 10.3 12 Qualified 7.5 0.09 10.1

Example 18 Stability of 10 mg/ml Composip (FP3 fusion protein) eye drops at 4 ° C

1、Preparation of 10 mg/ml Compaqep (FP3 fusion protein) eye drops according to Example 3.

2、The above 10 mg/ml Compaqip (FP3 fusion protein) eye drops were taken at 4 ° C, at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months. The samples were assayed and stability was determined by SEC-HPLC. The results show that the prescription can effectively inhibit the formation of polymer, the polymer in the product increases slowly, and the affinity of the fusion protein to VEGF is almost unchanged. The specific results are shown in Table 4.

Table 4. Stability of 10 mg/ml FP3 fusion protein at 4 °C

CN103212075B Eye drop containing VEGF antagonist

1.An eye drop containing a VEGF antagonist, characterized by containing

(a) 0.1-1 mg/ml of the fusion protein of SEQ ID No: 6;

(b) 10-50 mM citric acid;

(c) one or both of 50-100 mM arginine or histidine;

(d) 5-20% sucrose;

(e) 0.01 to 0.1% of a surfactant or cosolvent selected from one or both of polyethylene glycol or Tween 20;

(f) The pH is adjusted to 7.5 to 8.3.

2.The ophthalmic solution according to claim 1, characterized by comprising

(a) 0.5 mg/ml of the fusion protein of SEQ ID No: 6;

(b) 10 mM citric acid;

(c) 100 mM arginine;

(d) 5% sucrose;

(e) 0.05% Tween-20;

(f) The pH is adjusted to 7.5 to 8.3.

3.Use of the ophthalmic solution according to any one of claims 1 to 2 for the preparation of a medicament for treating an ocular surface disease caused by angiogenesis or growth.

4.The use of the ophthalmic solution according to claim 3 for the preparation of a medicament for treating an ocular surface disease caused by angiogenesis or growth, characterized in that the ocular surface disease is neovascularization, corneal neovascularization, and ocular surgery after corneal transplantation. Any of the neovascular or pterygium or its complications.

(19)中华人民共和国国家知识产权局

(12)发明专利

(10)授权公告号 CN 103212075 B
(45)授权公告日 2017.06.27

(21)申请号 201210017896.5

(22)申请日 2012.01.19

(65)同一申请的已公布的文献号
申请公布号 CN 103212075 A

(43)申请公布日 2013.07.24

(73)专利权人 成都康弘生物科技有限公司
地址 610036 四川省成都市金牛区蜀西路36号

(72)发明人 柯潇 郑强

(51)Int.Cl.
A61K 45/00(2006.01)
A61K 39/395(2006.01)
A61K 9/08(2006.01)
A61K 47/10(2006.01)
A61P 27/02(2006.01)
A61P 27/06(2006.01)

(56)对比文件
CN 102233132 A,2011.11.09,实施例2,说明书第13-15段,序列表.
CN 101478949 A,2009.07.08,权利要求1.

审查员 包宁疆

权利要求书1页 说明书12页
序列表8页

(54)发明名称

一种含有VEGF拮抗剂的滴眼液

(57)摘要

本发明涉及一种含有VEGF拮抗剂的滴眼液，该滴眼液中含有低浓度的VEGF拮抗剂，尤其是含有0.05-9.0mg/ml的VEGF拮抗剂，本发明滴眼液在确保安全稳定的基础上其疗效显著。

CN 103212075 B

1.一种含有VEGF拮抗剂的滴眼液,其特征在于含有

(a)0.1-1mg/ml的如SEQ ID No:6的融合蛋白;

(b)10-50mM的柠檬酸;

(c)50-100mM的精氨酸或组氨酸的一种或两种;

(d)5-20%的蔗糖;

(e)0.01-0.1%的表面活性剂或助溶剂,选自聚乙二醇或吐温20的一种或两种;

(f)调节pH为7.5~8.3。

2.根据权利要求1所述的滴眼液,其特征在于含有

(a)0.5mg/ml的如SEQ ID No:6的融合蛋白;

(b)10mM的柠檬酸;

(c)100mM的精氨酸;

(d)5%的蔗糖;

(e)0.05%的吐温-20;

(f)调节pH为7.5~8.3。

3.根据权利要求1-2中任一项所述的滴眼液在制备治疗由血管新生或生长引起的眼表疾病的药物中的用途。

4.根据权利要求3所述的滴眼液在制备治疗由血管新生或生长引起的眼表疾病的药物中的用途,其特征在于所述眼表疾病为角膜移植术后新生血管、角膜新生血管、眼表新生血管或翼状胬肉的任一种或其并发症。

一种含有VEGF拮抗剂的滴眼液

技术领域

[0001] 本发明涉及医药制剂领域，具体涉及一种含有VEGF拮抗剂的滴眼液。

背景技术

[0002] 角膜新生血管、新生血管性青光眼、翼状胬肉、慢性结膜炎等眼表病的发病均与新生血管的产生有一定的关系，VEGF的过度表达可以诱导眼表新生血管形成。其中角膜新生血管不是一种独立的角膜病，而是一种病理改变。导致角膜新生血管的原因很多，免疫炎症性、感染性、变性、外伤性、隐形眼镜的不当使用，医源性疾病等。上述原因导致的角膜新生血管如果不及时治疗，最终会导致角膜盲。角膜新生血管为世界范围视力丧失和失明的主要原因，在美国约4.14%的眼科患者患有角膜新生血管，美国每年有140万新增角膜新生血管患者，其中12%的会导致视力下降，在美国每年有约17万新增视力下降的角膜新生血管患者。中国尚没有关于角膜新生血管的流行病学调查，如果按照美国角膜新生血管占眼科患者4%，其中12%会导致视力下降来计算，2009年全国眼科患者为6283万，国内每年约有30万的新增患者因角膜新生而影响视力。患者角膜新生血管进展会导致视力的进一步恶化，最终需要角膜移植才能恢复视力。因此此类患者需要进行有效的抗新生血管治疗，以防止视力进一步丧失，同时避免进行角膜移植，医生对于角膜新生血管没有有效的治疗手段。

[0003] 生物药物，尤其是重组类蛋白许多特性完全不同于小分子化学分子，其发生不稳定降解反应是一个多步骤反应。限于目前分析方法的局限，发生于高级结构的这些饱含多个步骤的降解也很难精确测量到，其稳定性，尤其在常规条件下的保存就是一个很大的挑战。眼睛是人体最重要的器官之一：大脑的近80%的信息来自眼睛；同时眼睛由于其特殊的生理构造，是比较柔弱的组织，对粘度，渗透压等要求都非常高。因此对于眼用制剂，尤其是有外伤时候采用的眼用制剂要求异常严格。

[0004] VEGF(血管内皮细胞生长因子)的生物学效应均是通过其特异性受体VEGFR(血管内皮细胞生长因子受体)介导来实现的，VEGFR可导致由配体介导的二聚体化，受体的二聚体化促使相邻受体亚基自身磷酸化和去磷酸化，从而触发信号转导。VEGFR均为酪氨酸蛋白激酶，按其功能和结构分为：fins-样酪氨酸激酶-1(VEGFR-1/Flt-1)、激酶插入区受体(VEGFR-2/Flk-1/KDR)、fins-样酪氨酸激酶-4(VEGFR-3/Flt-4)及一些低相对分子质量VEGFR(neuropilin-1)。Flt-1和KDR主要分布于血管内皮细胞上，Flt-4则主要分布于淋巴管内皮细胞上。与VEGF相结合的主要为Flt-1和KDR，Flt-1与VEGF的结合力较KDR高，他们都是糖基化的跨膜受体，直接参与VEGF进入细胞内的信号传递。Flt-1与VEGF结合后能促使血管内皮细胞的形成和调节血管渗透性；KDR与VEGF结合后则能促进血管内皮细胞的增生及成熟。VEGF拮抗剂，尤其是一类人工设计的靶向基因工程蛋白，如Lucentis、Avastin、VEGF-trap等等，它们能有效地阻断由血管内皮生长因子(VEGF)介导的信号传递，抑制病变新生血管的生长，并用于治疗肿瘤、眼睛等由新生血管引起的疾病，但是上述药物的已上市的制剂形式为注射剂，其给药方式为玻璃体直接注射或全身给药，其制剂形式对于患者而言，其依从性差，且存在诸多不便。因此，研究者开始研究一种患者依从性好，且方便有效的含有

VEGF拮抗剂的药物制剂形式，US7303748公开了一种含有VEGF-trap的滴眼液，其制剂处方：39.4-103.06mg/ml VEGF-trap，5mM磷酸，5mM柠檬酸，100mM氯化钠，0.005％吐温-20。但是该滴眼液浓度较大，由于生物制剂一般都存在不稳定的问题，目前眼表的抗血管治疗患者很多都不愿住院治疗，高浓度的生物制剂很容易造成保存和使用的不便，同时眼表给药是一个特殊的给药途径，对pH、渗透压和局部刺激性，不能添加抑菌剂和抗菌剂等，上述问题使得用于眼表抗新生血管治疗药物的成本昂贵，效果有限。因此，提供一种浓度低，抑制新生血管疗效确切的滴眼液，并将其用于治疗角膜新生血管、新生血管性青光眼、翼状胬肉、慢性结膜炎等多种眼表疾病，对于满足临床需求具有显著的意义。

发明内容

[0005] 本发明需要解决技术问题之一是提供一种浓度小但是疗效确切的含有VEGF拮抗剂的滴眼液。

[0006] 为了解决上述技术问题，本发明提供了如下技术方案：

[0007] 本发明一方面提供了一种含有VEGF拮抗剂的滴眼液，该滴眼液中含有0.05-9.0mg/ml的VEGF拮抗剂；优选含有0.1-1.0mg/ml的VEGF拮抗剂；更优选含有0.5mg/ml的VEGF拮抗剂；其中所述VEGF拮抗剂优选为含有FLT-1和KDR的片断的融合蛋白。

[0008] 上述VEGF拮抗剂优选具有如下结构之一的融合蛋白：

[0009] a.FP1，由FLT-1的第2免疫球蛋白样区域和KDR的第3免疫球蛋白样区域与人免疫球蛋白Fc片段融合而成的蛋白：FLTd2-KDRd3-Fc；

[0010] b.FP2，由KDR的第1免疫球蛋白样区域，FLT-1的第2免疫球蛋白样区域和KDR的第3免疫球蛋白样区域与人免疫球蛋白Fc片段融合而成的蛋白：

[0011] KDRd1-FLTd2-KDRd3-Fc；

[0012] c.FP3，由FLT-1的第2免疫球蛋白样区域和KDR的第3-4免疫球蛋白样区域与人免疫球蛋白Fc片段融合而成的蛋白：FLTd2-KDRd3,4-Fc；

[0013] d.FP4，由FLT-1的第2免疫球蛋白样区域，KDR的第3免疫球蛋白样区域和FLT-1的第4免疫球蛋白样区域与人免疫球蛋白Fc片段融合而成的蛋白：

[0014] FLTd2-KDRd3-FLTd4-Fc；

[0015] e.FP5，由FLT-1的第2免疫球蛋白样区域和KDR的第3-5免疫球蛋白样区域与人免疫球蛋白Fc片段融合而成的蛋白：FLTd2-KDRd3,4,5-Fc；或

[0016] f.FP6，由FLT-1的第2免疫球蛋白样区域，KDR的第3免疫球蛋白样区域和FLT-1的第4-5免疫球蛋白样区域与人免疫球蛋白Fc片段融合而成的蛋白：

[0017] FLTd2-KDRd3-FLTd4,5-Fc；

[0018] g.FP7，由FLT-1的第2免疫球蛋白样区域和KDR的第3-4免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3,4；

[0019] h.FP8，由FLT-1的第2免疫球蛋白样区域和KDR的第3免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3。

[0020] 以上FLT-1和KDR免疫球蛋白样区域的氨基酸FLT-1D2、FLT-1D4、KDRD1、KDRD3、KDRD4见序列表1-5，FP3蛋白的氨基酸序列见序列表6、FP1蛋白的氨基酸序列见序列表7，FP7蛋白的氨基酸序列见序列表8，FP8蛋白的氨基酸序列见序列表9。

[0021] 本发明提供的滴眼液中，还可以含有以下组分的一种或多种：

[0022] (a) 5-100mM缓冲液，其中的酸选自Tris-HCl，柠檬酸，磷酸、磷酸氢钠，磷酸二氢钠，醋酸，丁二酸，盐酸中的一种或多种；

[0023] (b) 5-500mM碱性氨基酸选自赖氨酸，精氨酸，和组氨酸中的一种或其组合；

[0024] (c) 0.1-30％盐渗透压剂调节剂，其中的糖选自蔗糖，海藻糖，甘露醇，甘油，丙二醇，山梨酯醇中的一种或多种，盐选自氯化钠或其它药学上可以接受的盐中的一种或其组合；

[0025] (d) 0.005-0.1％的一种或多种表面活性剂或助溶剂，选自聚乙二醇，吐温20，吐温80，丙二醇，二甲基亚砜或其它药学上可以接受的表面活性剂中的一种或多种；

[0026] 上述组分之一或多种配成的溶液pH为6～8.3。

[0027] 上述滴眼液中所述的VEGF拮抗剂最优选为如SEQ ID No:6的融合蛋白。

[0028] 本发明还进一步提供了含有如下组分的滴眼液，具体组分如下：

[0029] (a) 0.05-9mg/ml的如SEQ ID No:6的融合蛋白；

[0030] (b) 5-250mM的柠檬酸；

[0031] (c) 5-500mM的精氨酸或组氨酸的一种或两种；

[0032] (d) 4-30％的蔗糖或海藻糖；

[0033] (e) 0.01-0.1％的表面活性剂或助溶剂，选自聚乙二醇或吐温20的一种或两种；

[0034] (f) 调节pH为7.5～8.3。

[0035] 本发明再进一步提供了含有如下组分的滴眼液，具体组分如下：

[0036] (a) 0.1-1mg/ml的如SEQ ID No:6的融合蛋白；

[0037] (b) 10-50mM的柠檬酸；

[0038] (c) 50-100mM的精氨酸或组氨酸的一种或两种；

[0039] (d) 5-20％的蔗糖；

[0040] (e) 0.01-0.1％的表面活性剂或助溶剂，选自聚乙二醇或吐温20的一种或两种；

[0041] (f) 调节pH为7.5～8.3。

[0042] 本发明更进一步提供了含有如下组分的滴眼液，具体组分如下：

[0043] (a) 0.5mg/ml的如SEQ ID No:6的融合蛋白；

[0044] (b) 50mM的柠檬酸；

[0045] (c) 250mM的精氨酸；

[0046] (d) 12.5％的蔗糖；

[0047] (e) 0.05％的吐温-20；

[0048] (f) 调节pH为7.5～8.3。

[0049] 本发明还提供了上述滴眼液在制备治疗由血管新生或生长引起的眼表疾病的药物中的用途；优选为眼表疾病为角膜移植术后新生血管、角膜新生血管、眼表新生血管或翼状胬肉的任一种或其并发症。

[0050] 中国专利ZL200510073595.4和ZL200610066257.2公开的技术内容作为本申请的参考；中国未公开专利CN 201010267503.7的技术内容也作为本申请的参考。

[0051] 本发明与现有技术相比其优势在于：滴眼液中融合蛋白的含量极低、且疗效确切，在低浓度滴眼液较高的稳定性的基础上能够大幅度节约生产成本。

具体实施方式

[0052] 以下实施例仅作为对本发明的进一步解释，不能作为是对本发明保护范围的限制。

[0053] 实施例1、康柏西普滴眼液对碱烧伤致角膜新生血管研究

[0054] 药品：盐酸金霉素眼膏，规格批号：2.0g/支，411002，有效期至2014.12，重庆科瑞制药有限责任公司；盐酸利多卡因注射液，规格批号：5ml/支，0.1g/支，有效期至2012.04.，天津药物焦作有限公司。

[0055] 试剂：氢氧化钠(NaOH)，规格批号：500g/瓶，20091223，成都市科龙化工试剂厂。

[0056] 受试样品：

[0057] A，康柏西普滴眼液(按照实施例3制备)10mg/ml，无色透明液体，1ml/支，批号：FR1108001，保存于2～8℃，使用时滴加入眼睛表面；

[0058] B，康柏西普滴眼液(按照实施例3制备)9mg/ml，无色透明液体，1ml/支，批号：FR1108002，保存于2～8℃，使用时滴加入眼睛表面；

[0059] C，地塞米松，无色透明液体，1ml/支，批号：FR1108003，保存于2～8℃，使用时滴加入眼睛表面；

[0060] D，康柏西普滴眼液(按照实施例3制备)0.1mg/ml，无色透明液体，1ml/支，批号：FR1108004，保存于2～8℃，使用时滴加入眼睛表面；

[0061] E，不含康柏西普制剂缓冲液(buffer)(按照实施例16制备)，无色透明液体，1ml/支，批号：FR1108005，保存于2～8℃，使用时滴加入眼睛表面；

[0062] F，康柏西普滴眼液(按照实施例3制备)5mg/ml，无色透明液体，1ml/支，批号：FR1108006，保存于2～8℃，使用时滴加入眼睛表面；

[0063] 均由康弘药业集团企业技术中心中化药研究部制剂室提供。

[0064] 实验方法及结果：

[0065] 取健康无眼疾病新西兰兔48只，称其体重，用3%戊巴比妥钠麻醉(1ml/kg)，并于眼睛表面施予局麻药盐酸利多卡因液，剂量为20μl/眼；制备9mm直径滤纸片，浸泡于1mol/LNaOH溶液中约10s，用镊子将滤纸片放置于干燥的滤纸上吸去多余的NaOH溶液，将浸有NaOH的滤纸片置于家兔双眼眼角膜正中60s后取下，迅速取洗瓶用约20ml生理盐水冲洗角膜，并给予预防抗生素防止感染(盐酸金霉素眼膏)，3次/2天。

[0066] 造模后随即分为烧伤、A(康柏西普滴眼液(按照实施例3制备)10mg/ml)、B(康柏西普滴眼液(按照实施例3制备)9mg/ml)、C(地塞米松)、D(康柏西普滴眼液(按照实施例3制备)0.1mg/ml)、E(不含康柏西普的制剂缓冲溶液(按照实施例16制备))和F(康柏西普滴眼液(按照实施例3制备)5mg/ml)七个组，造模当天为0天，从第1天开始，A～F组给予相应药物，给药频率6次/天，给药剂量每次50μl/眼，连续给药10天，另取2只新西兰兔设为正常组。每天给药同时，观察眼角膜NV生长状态及眼部是否有炎症反应。

[0067] 于给药第10天，以3%戊巴比妥钠溶液麻醉动物(1ml/kg)，并于眼睛表面施予局麻药盐酸利多卡因液，剂量为20μl/眼，在裂隙灯10倍物镜下观察兔眼角膜NV钟点方向，同时在10倍和16倍物镜下照相。采集图像在Photoshop CS进行钟点数校正，角膜新生面积采用Image Pro Plus处理；面积公式：$S=C/12\times3.1416\times[R^2-(R-L)^2]$，C表示在图片中角膜边缘

从有NV到无NV生长时点所占的钟点数，R表示在图片中从角膜与巩膜接触的边缘到角膜中心的长度，L表示在图片中从角膜与巩膜接触边缘NV的根部到角膜中NV的末端NV长度，每个钟点中取最长的一根血管长度。所有数据统计分析采用T检验方差分析。数据以$\bar{x}\pm s$表示，是平均值，s是标准偏差。

[0068] 通过一般眼表观察发现，烧伤第1，2天各组烧伤眼角膜边缘血管网充血明显；烧伤第3天，角膜边缘有NV生长；第5天康柏西普滴眼液5mg/ml和不含康柏西普的制剂缓冲溶液眼角膜表面NV生长明显，地塞米松、康柏西普滴眼液10mg/ml、康柏西普滴眼液9mg/ml和康柏西普滴眼液0.1mg/ml组NV生长不明显；第7天各组眼角膜表面NV生长明显，烧伤组和不含康柏西普的制剂缓冲溶液组角膜NV向烧伤角膜生长，部分NV已生长到烧伤角膜边缘，而地塞米松、康柏西普滴眼液10mg/ml、康柏西普滴眼液9mg/ml、康柏西普滴眼液5mg/ml和康柏西普滴眼液0.1mg/ml组很少有NV生长到烧伤角膜边缘；第10天时烧伤组和不含康柏西普的制剂缓冲溶液组已有NV长入烧伤角膜内，而地塞米松、康柏西普10mg/ml、康柏西普滴眼液9mg/ml、康柏西普5mg/ml和康柏西普0.1mg/ml组NV长入烧伤角膜较少。

[0069] 角膜新生血管面积影响：第10天角膜NV面积数据分析结果可知，与碱烧伤组和不含康柏西普的制剂缓冲溶液组相比，地塞米松、康柏西普10mg/ml、康柏西普滴眼液9mg/ml、康柏西普5mg/ml和康柏西普0.1mg/ml能够明显抑制角膜NV生长，减少NV面积，具有统计学意义($P<0.05$)，其中地塞米松与康柏西普0.1mg/ml抑制NV生长最佳($P<0.01$)。虽然不含康柏西普的制剂缓冲溶液无抑制角膜新生血管生长作用，且NV面积大于烧伤组，但无显著性差异。结果如表1。

[0070] 表1各药物对碱烧伤致兔眼角膜NV的影响($\bar{x}\pm s$)

[0071]

组别	眼睛数	药物剂量（mg/ml）	给药体积(μl)	NV 面积(mm^2)
正常组	2	—	—	0
烧伤组	10	——	——	31.08±6.091
不含康柏西普的制剂 buffer E	11	0	50	46.271±33.461
地塞米松 C	11	0.25	50	15.367± 9.698$^{**\#\#\wedge\wedge}$
康柏西普滴眼液 A	11	10	50	20.16±13.829$^{*\#\wedge}$
康柏西普滴眼液 F	11	9	50	20.24±12.670$^{*\#\wedge}$
康柏西普滴眼液 B	10	5	50	20.618±14.073$^{*\#\wedge}$
康柏西普滴眼液 D	11	0.1	50	16.995± 6.464$^{**\#\#\wedge}$

[0072] 注：阴性对照为不含药物的缓冲液，阳性对照为地塞米松，与烧伤组比较，$^{*}P<0.05$，$^{**}P<0.01$；与B组比较，$^{\#}P<0.05$，$^{\#\#}P<0.01$；与E组比较，$^{\wedge}P<0.05$，$^{\wedge\wedge}P<0.01$。

[0073] 实施例2低剂量康柏西普滴眼液对碱烧伤致角膜NV生长影响

[0074] 药品：盐酸金霉素眼膏，规格批号：2.0g/支，411002，有效期至2014.12，重庆科瑞

制药有限责任公司；盐酸利多卡因注射液，规格批号：5ml/支，0.1g/支，有效期至2012.04.，天津药物焦作有限公司。

[0075] 试剂：氢氧化钠(NaOH)，规格批号：500g/瓶，20091223，成都市科龙化工试剂厂。

[0076] 受试样品：

[0077] A，康柏西普滴眼液(按照实施例3制备)0.5mg/ml，无色透明液体，800μl/支，批号：20111001，保存于2～8℃，使用时滴加入眼睛表面；

[0078] B，康柏西普滴眼液(按照实施例3制备)0.1mg/ml，无色透明液体，800μl/支，批号：20111001，保存于2～8℃，使用时滴加入眼睛表面；

[0079] C，不含康柏西普的制剂缓冲溶液，无色透明液体，800μl/支，批号：20111001，保存于2～8℃，使用时滴加入眼睛表面；

[0080] D，康柏西普滴眼液(按照实施例3制备)0.01mg/ml，无色透明液体，800μl/支，批号：20111001，保存于2～8℃，使用时滴加入眼睛表面；

[0081] E，地塞米松，无色透明液体，800μl/支，批号：20111001，保存于2～8℃，使用时滴加入眼睛表面；

[0082] F，康柏西普滴眼液(按照实施例3制备)0.05mg/ml，无色透明液体，800μl/支，批号：20111001，保存于2～8℃，使用时滴加入眼睛表面；

[0083] G，康柏西普滴眼液(按照实施例3制备)1mg/ml，无色透明液体，800μl/支，批号：20111001，保存于2～8℃，使用时滴加入眼睛表面；

[0084] 均由康弘药业集团企业技术中心中化药研究部制剂室提供。

[0085] 实验方法及结果：

[0086] 试验分为A(康柏西普滴眼液(按照实施例3制备)0.5mg/ml)、B(康柏西普滴眼液(按照实施例3制备)0.1mg/ml)、C(不含康柏西普的制剂缓冲液(按照实施例16制备)buffer)、D(康柏西普滴眼液(按照实施例3制备)0.01mg/ml)、E(地塞米松)、F(康柏西普滴眼液(按照实施例3制备)0.05mg/ml)和G(康柏西普滴眼液(按照实施例3制备)1mg/ml)八个组。

[0087] 试验方法操作同实施例1。

[0088] 通过眼表观察发现，烧伤第1，2天各组烧伤眼角膜边缘血管网充血明显，部分眼睑红肿现象；烧伤第3天，角膜边缘有NV生长趋势，眼睑红肿消退；第5天，各组角膜表面有NV生长，但是有部分兔眼睑又出现红肿现象；第7天各组眼角膜表面NV生长明显，有明显地向心性生长，而地塞米松组角膜NV较少，但兔眼睑红肿现象更加严重；第10天，含有康柏西普的各组角膜NV生长明显，部分角膜新生血管已长入烧伤部位，而地塞米松组NV长入角膜烧伤部位较少，兔眼睑红肿现象未见恢复，而含有康柏西普的各组未有兔眼睑红肿现象。

[0089] 角膜新生血管面积影响：第10天角膜NV面积数据分析结果可知，与不含康柏西普的制剂缓冲溶液组相比，地塞米松、康柏西普滴眼液0.5mg/ml能够明显抑制角膜NV生长，减少NV面积，具有统计学意义($P<0.05$)。康柏西普滴眼液1mg/ml、康柏西普滴眼液0.1mg/ml也能抑制角膜NV生长。而康柏西普滴眼液0.01mg/ml无抑制角膜新生血管生长作用，且NV面积大于烧伤组，但无显著性差异。见表2。

[0090] 表2各药物对碱烧伤致兔眼角膜NV的影响($\bar{x} \pm s$)

[0091]

组别	眼睛数	药物剂量（mg/ml）	给药体积(μl)	NV 面积(mm^2)	眼睛红肿数
正常组	1	——	——	0	0
不含康柏西普的制剂缓冲液（buffer）C	10	0	50	35.16±17.614	1
阴性对照:地塞米松 E	9	0.25	50	12.65±12.385**	4
康柏西普滴眼液 G	12	1	50	24.56±15.498	1
康柏西普滴眼液 A	12	0.5	50	20.10±9.643*	0
康柏西普滴眼液 B	12	0.1	50	26.31±13.630	0
康柏西普滴眼液 F	12	0.05	50	28.09±20.253	0
康柏西普滴眼液 D	12	0.01	50	40.75±18.793	0

[0092] 注:Buffer对照,*P＜005,**P＜001。

[0093] 实施例3、康柏西普(FP3融合蛋白)滴眼液的制备

[0094] 处方:

[0095] FP3融合蛋白10mg/ml、9mg/ml、5mg/ml、1mg/ml、0.5mg/ml、0.1mg/ml、0.05mg/ml或0.01mg/ml

[0096]

柠檬酸	55 mM
蔗糖	12.5 %
精氨酸	250 mM
吐温 20	0.05 %

[0097] pH 7.5~8.3

[0098] 制备方法:将浓缩换液后的FP3融合蛋白原液化冻后,在C级洁净去洁净台(无菌柜)中,按无菌操作法加入过滤除菌的含有55mM柠檬酸、12.5%蔗糖、250mM精氨酸和0.05%吐温20的缓冲液、滤过、分别调节FP3融合蛋白至10mg/ml、9mg/ml、5mg/ml、1mg/ml、0.5mg/ml、0.1mg/ml、0.05mg/ml或0.01mg/ml,pH为7.5～8.3,无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0099] 实施例4、康柏西普(FP3融合蛋白)滴眼液的制备

[0100] 处方:FP3 融合蛋白 0.5 mg/ml

[0101]

柠檬酸	10 mM
蔗糖	8.0 %
精氨酸	5 mM
吐温 20	0.05 %
pH	7.5~8.3

[0102] 制备方法:将浓缩换液后的FP3融合蛋白原液化冻后,在C级洁净去洁净台(无菌

柜)中,按无菌操作法加入过滤除菌的含有10mM柠檬酸、8.0%蔗糖、5mM精氨酸和0.05%吐温20的缓冲液,滤过、调节FP3融合蛋白至0.5mg/ml,pH为7.5～8.3,无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0103] 实施例5康柏西普(FP3融合蛋白)滴眼液的制备

[0104] 处方:

[0105]

FP3 融合蛋白	1.0 mg/ml
柠檬酸	100 mM
蔗糖	20.0 %
精氨酸	250 mM
吐温 20	0.10 %
pH	7.5~8.3

[0106] 制备方法:将浓缩换液后的FP3融合蛋白原液化冻后,在C级洁净去洁净台(无菌柜)中,按无菌操作法加入过滤除菌的含有100mM柠檬酸、20.0%蔗糖、250mM精氨酸和0.10%吐温20的缓冲液,滤过,调节FP3融合蛋白至1.0mg/ml,pH为7.5～8.3无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0107] 实施例6康柏西普(FP3融合蛋白)滴眼液的制备

[0108] 处方:

[0109]

FP3 融合蛋白	0.1mg/ml
磷酸二氢钠	5 mM
海藻糖	10.0 %
精氨酸	100 mM
PEG400	0.01 %
pH	7.5~8.3

[0110] 制备方法:将浓缩换液后的FP3融合蛋白原液化冻后,在C级洁净去洁净台(无菌柜)中,按无菌操作法加入过滤除菌的含有5mM磷酸二氢钠、10.0%海藻糖、100mM精氨酸和0.01%PEG400的缓冲液,滤过,调节FP3融合蛋白至0.1mg/ml,pH为7.5～8.3,无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0111] 实施例7康柏西普(FP3融合蛋白)滴眼液的制备

[0112] 处方:

FP3 融合蛋白 0.05 mg/ml

柠檬酸 5 mM

蔗糖 4.0 %

氯化钠 4.0%

[0113] 精氨酸 100 mM

组氨酸 100 mM

吐温 20 0.05 %

PEG400 0.05%

pH 7.5~8.3

[0114] 制备方法：将浓缩换液后的FP3融合蛋白原液化冻后，在C级洁净去洁净台（无菌柜）中，按无菌操作法加入过滤除菌的含有5mM柠檬酸、4.0％蔗糖、4.0％氯化钠、100mM精氨酸、100mM组氨酸、0.05％吐温20、0.05％PEG400的缓冲液，滤过，调节FP3融合蛋白至0.05mg/ml、pH为7.5～8.3，无菌分装到盛装滴眼液的容器中，存放于2-8℃。

[0115] 实施例8康柏西普（FP3融合蛋白）滴眼液的制备

[0116] 处方：

FP3 融合蛋白 5.0 mg/ml

柠檬酸 10 mM

[0117]

蔗糖 30 %

精氨酸 500 mM

吐温 20 0.1 %

[0118]

pH 7.5~8.3

[0119] 制备方法：将浓缩换液后的FP3融合蛋白原液化冻后，在C级洁净去洁净台（无菌柜）中，按无菌操作法加入过滤除菌的含有10mM柠檬酸、30.0％蔗糖、500mM精氨酸、0.1％吐温20的缓冲液，滤过，调节FP3融合蛋白至5.0mg/ml、pH为7.5～8.3，无菌分装到盛装滴眼液的容器中，存放于2-8℃。

[0120] 实施例9康柏西普（FP3融合蛋白）滴眼液的制备

[0121] 处方：

FP3 融合蛋白 0.5 mg/ml

柠檬酸 10 mM

蔗糖 5 %

[0122]

精氨酸 100 mM

吐温 20 0.05 %

pH 7.5~8.3

[0123] 制备方法：将浓缩换液后的FP3融合蛋白原液化冻后，在C级洁净去洁净台（无菌

柜)中,按无菌操作法加入过滤除菌的含有10mM柠檬酸、5.0%蔗糖、100mM精氨酸、0.05%吐温20的缓冲液、滤过,调节FP3融合蛋白至0.5mg/ml、pH为7.5~8.3,无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0124] 实施例10康柏西普(FP3融合蛋白)滴眼液的制备

[0125] 处方:

[0126]

FP3 融合蛋白	0.5 mg/ml
柠檬酸	250 mM
蔗糖	8.0 %
组氨酸	100 mM
吐温 20	0.10 %
pH	7.5~8.3

[0127] 制备方法:将浓缩换液后的FP3融合蛋白原液化冻后,在C级洁净去洁净台(无菌柜)中,按无菌操作法加入过滤除菌的含有250mM柠檬酸、8.0%蔗糖、100mM组氨酸、0.10%吐温20的缓冲液、滤过,调节FP3融合蛋白至0.5mg/ml、pH为7.5~8.3,无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0128] 实施例13康柏西普(FP3融合蛋白)滴眼液的制备

[0129] 处方:

[0130]

FP3 融合蛋白	0.5 mg/ml
柠檬酸	10 mM
蔗糖	5 %
精氨酸	100 mM
吐温 20	0.05 %
pH	7.5~8.3

[0131] 制备方法:将浓缩换液后的FP3融合蛋白原液化冻后,在C级洁净去洁净台(无菌柜)中,按无菌操作法加入过滤除菌的含有10mM柠檬酸、5.0%蔗糖、100mM精氨酸、0.05%吐温20的缓冲液、滤过,调节FP3融合蛋白至0.5mg/ml、pH为7.5~8.3,无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0132] 实施例14系列融合蛋白滴眼液的制备

[0133] 处方:

融合蛋白 9 mg/ml、5 mg/ml、1 mg/ml、0.5 mg/ml、0.1 mg/ml 或 0.05 mg/ml

柠檬酸 55 mM

蔗糖 12.5 %

[0134]

精氨酸 250 mM

吐温 20 0.05 %

pH 7.5~8.3

[0135] 制备方法：将浓缩换液后的各种融合蛋白（FP1、FP2、FP4、FP5、FP6、FP7、FP8）原液化冻后，在C级洁净去洁净台（无菌柜）中，按无菌操作法加入过滤除菌的含有55mM柠檬酸、12.5%蔗糖、250mM精氨酸和0.05%吐温20的缓冲液、过滤，分别调节融合蛋白至9mg/ml、5mg/ml、1mg/ml、0.5mg/ml、0.1mg/ml或0.05mg/ml，pH为6.0～8.3，无菌分装到盛装滴眼液的容器中，存放于2-8℃。

[0136] 实施例15FP1融合蛋白滴眼液的制备

[0137] 处方：

FP1 融合蛋白 1.0 mg/ml，

磷酸 5 mM

[0138] 柠檬酸 5 mM

氯化钠 100 mM，

吐温-20 0.005%

[0139] pH 6.0

[0140] 制备方法：将浓缩换液后的FP1融合蛋白原液化冻后，在C级洁净去洁净台（无菌柜）中，按无菌操作法加入过滤除菌的含有5mM磷酸、5mM柠檬酸、100mM氯化钠和0.005%吐温20的缓冲液、滤过，调节融合蛋白至1.0mg/ml，pH为6.0-8.0，无菌分装到盛装滴眼液的容器中，存放于2-8℃。

[0141] 实施例16不含康柏西普（FP3融合蛋白）的制剂缓冲液制备

[0142] 处方：

柠檬酸 55 mM

蔗糖 12.5 %

[0143] 精氨酸 250 mM

吐温 20 0.05 %

pH 7.5~8.3

[0144] 制备方法：将按无菌操作法加入过滤除菌的含有55mM柠檬酸、12.5%蔗糖、250mM精氨酸和0.05%吐温20的缓冲液、滤过、分别调节pH为7.5～8.3，无菌分装到盛装滴眼液的容器中，存放于2-8℃。

[0145] 实施例17 0.1mg/ml康柏西普（FP3融合蛋白）滴眼液在25℃的稳定性

[0146] 1、按照实施例3制备0.1mg/ml康柏西普（FP3融合蛋白）滴眼液

[0147] 2、将上述0.1mg/ml康柏西普(FP3融合蛋白)滴眼液,25℃留样,在0,1,2,3,4,5,6,7,8,9,10,11,12月测定样品,通过SEC-HPLC确定稳定性。结果显示,该处方能有效抑制聚合物的生成,产品纯度基本不下降,融合蛋白与VEGF的亲和力几乎不变。

[0148] 具体结果见表3。

[0149] 表3.0.1mg/ml FP3融合蛋白在25℃的稳定性

[0150]

时间(月)	外观	pH值	聚合物(%)	亲和力(pM)
0	合格	7.2	0.01	10.6
1	合格	7.4	0.03	10.5
2	合格	7.5	0.03	10.4
3	合格	7.5	0.05	10.4
6	合格	7.7	0.07	10.2
9	合格	7.6	0.08	10.3
12	合格	7.5	0.09	10.1

[0151] 实施例18 10mg/ml康柏西普(FP3融合蛋白)滴眼液在4℃的稳定性

[0152] 1、按照实施例3制备10mg/ml康柏西普(FP3融合蛋白)滴眼液

[0153] 2、将上述10mg/ml康柏西普(FP3融合蛋白)滴眼液,4℃留样,在0,1,2,3,4,5,6,7,8,9,10,11,12月测定样品,通过SEC-HPLC确定稳定性。结果显示,该处方能有效抑制聚合物的生成,产品中聚合物增加缓慢,融合蛋白与VEGF的亲和力几乎不变。具体结果见表4。

[0154] 表4.10mg/ml FP3融合蛋白在4℃的稳定性

[0155]

时间（月）	外观	pH 值	浓度（mg/ml）	聚合物（%）	亲和力（pM）
0	合格	8.3	10.0	0.2	9.7
1	合格	8.3	10.1	0.2	9.6
2	合格	8.2	10.2	0.3	9.6
3	合格	7.9	10.0	0.4	9.3
6	合格	8.0	10.1	0.5	9.3
9	合格	7.9	10.1	0.6	9.1
12	合格	7.9	10.3	1.0	9.4

序列表

<110> 成都康弘生物科技有限公司
<120> 一种含有 VEGF 拮抗剂的滴眼液
<130> ZL200610066257.2
<160> 9
<170> PatentIn version 3.3
<210> 1
<211> 93
<212> PRT
<213> FLT-1D2
<400> 1
Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr
1 5 10 15
Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile
20 25 30
Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly
35 40 45
Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala
50 55 60
Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly
65 70 75 80
His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr
85 90

[0001]

<210> 2
<211> 96
<212> PRT
<213> FLT-1D4
<400> 2
Phe Ile Thr Val Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala
1 5 10 15
Gly Lys Arg Ser Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser
20 25 30
Pro Glu Val Val Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser
35 40 45
Ala Arg Tyr Leu Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr
50 55 60
Glu Glu Asp Ala Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser
65 70 75 80
Asn Val Phe Lys Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro
85 90 95
<210> 3
<211> 86
<212> PRT

<213> KDRD1
<400> 3
Pro Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr
1 5 10 15
Thr Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp
20 25 30
Pro Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys
35 40 45
Ser Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly
50 55 60
Asn Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala
65 70 75 80
Ser Val Ile Tyr Val Tyr
85

<210> 4
<211> 102
<212> PRT
<213> KDR D3
<400> 4
Val Val Leu Ser Pro Ser His Gly Ile Ile Glu Glu Leu Ser Val Gly
1 5 10 15
Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly
20 25 30
Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys
35 40 45
Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys
50 55 60
Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly
65 70 75 80
Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser
85 90 95
Thr Phe Val Arg Val His
100

[0002]

<210> 5
<211> 92
<212> PRT
<213> KDR D4
<400> 5
Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu Val Glu Ala Thr Val
1 5 10 15
Gly Glu Arg Val Arg Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro Pro
20 25 30
Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr
35 40 45

Ile Lys Ala Gly His Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp
50 55 60
Thr Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro Ile Ser Lys Glu Lys
65 70 75 80
Gln Ser His Val Val Ser Leu Val Val Tyr Val Pro
85 90

<210> 6
<211> 552
<212> PRT
<213> 人工序列
<400> 6

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
1 5 10 15
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu
20 25 30
Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu
35 40 45
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu
50 55 60
Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
65 70 75 80

[0003]

Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu
85 90 95
Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys
100 105 110
Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val
115 120 125
Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val
130 135 140
Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn
145 150 155 160
Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg
165 170 175
Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr
180 185 190
Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys
195 200 205
Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg
210 215 220
Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu
225 230 235 240
Val Glu Ala Thr Val Gly Glu Arg Val Arg Leu Pro Ala Lys Tyr Leu
245 250 255

Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Leu
260 265 270
Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr Ile Met Glu
275 280 285
Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro
290 295 300
Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val Val Tyr Val
305 310 315 320
Pro Pro Gly Pro Gly Asp Lys Thr His Thr Cys Pro Leu Cys Pro Ala
325 330 335
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
340 345 350
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
355 360 365
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
370 375 380
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
385 390 395 400
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
405 410 415
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
[0004] 420 425 430
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
435 440 445
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
450 455 460
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
465 470 475 480
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
485 490 495
Lys Ala Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
500 505 510
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
515 520 525
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
530 535 540
Ser Leu Ser Leu Ser Pro Gly Lys
545 550

<210> 7
<211> 455
<212> PRT
<213> 人工序列

<400> 7

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
1 5 10 15
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu
20 25 30
Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu
35 40 45
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu
50 55 60
Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
65 70 75 80
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu
85 90 95
Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys
100 105 110
Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val
115 120 125
Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val
130 135 140
Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn
145 150 155 160

[0005] Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg
165 170 175
Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr
180 185 190
Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys
195 200 205
Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg
210 215 220
Val His Glu Lys Asp Lys Thr His Thr Cys Pro Leu Cys Pro Ala Pro
225 230 235 240
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400
Ala Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445
Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 8
<211> 308
<212> PRT
<213> 人工序列
<400> 8

[0006]

Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His
1 5 10 15
Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro
20 25 30
Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro
35 40 45
Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser
50 55 60
Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val
65 70 75 80
Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn
85 90 95
Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser
100 105 110
Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn
115 120 125
Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His
130 135 140
Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met
145 150 155 160
Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp
165 170 175

Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys
180 185 190
Asn Ser Thr Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly
195 200 205
Ser Gly Met Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg
210 215 220
Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr
225 230 235 240
Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His
245 250 255
Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr
260 265 270
Val Ile Leu Thr Asn Pro Ile Ser Lys Glu Lys Glu Ser His Val Val
275 280 285
Ser Leu Val Val Tyr Val Pro Pro Asp Lys Thr His Thr Cys Pro Leu
290 295 300
Cys Pro Ala Pro
305

[0007]

<210> 9
<211> 214
<212> PRT
<213> 人工序列
<400> 9
Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His
1 5 10 15
Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro
20 25 30
Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro
35 40 45
Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser
50 55 60
Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val
65 70 75 80
Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn
85 90 95
Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser
100 105 110
Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn
115 120 125
Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His
130 135 140
Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met
145 150 155 160

[0008]

Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp
165 170 175
Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys
180 185 190
Asn Ser Thr Phe Val Arg Val His Glu Lys Asp Lys Thr His Thr Cys
195 200 205
Pro Leu Cys Pro Ala Pro
210

Espacenet

CN107115294A VEGF antagonist-containing eye drops

Applicants: CHENGDU KANGHONG BIOLOGICAL TECH CO LTD

Inventors: KE XIAO,ZHENG QIANG

Classifications:

IPC ***A61K39/395; A61K47/12; A61K47/18; A61K47/26; A61K9/08; A61P27/02;***

CPC **A61K39/3955 (CN); A61K47/12 (CN); A61K47/183 (CN); A61K47/26 (CN); A61K9/0048 (CN); A61K9/08 (CN);**

Priorities: CN201210017896A 2012-01-19; CN201710357021A 2012-01-19

Application: CN201710357021A·2012-01-19

Publication: CN107115294A·2017-09-01

Published as: **CN103212075A; CN103212075B;** CN107115294A;**CN107115294B**

VEGF antagonist-containing eye drops

Abstract

The invention relates to VEGF antagonist-containing eye drops which is prepared from a VEGF antagonist with low concentration, particularly a 0.05-9.0mg/ml VEGF antagonist. The eye drops provided by the invention is remarkable in curative effect on the basis of ensuring safety and stability.

CN107115294A VEGF antagonist-containing eye drops

This application is a divisional application with application number CN201210017896.5, filed January 19, 2012, entitled "Eye Drops Containing a VEGF Antagonist," filed on January 19, 2012.

Technical field

This invention relates to the field of pharmaceutical preparations, in particular to an ophthalmic solution containing a VEGF antagonist.

Background technique

Corneal neovascularization, neovascular glaucoma, pterygium, chronic conjunctivitis and other ocular surface disease are associated with the generation of neovascularization, VEGF overexpression can induce ocular neovascularization. Corneal neovascularization is not an independent corneal disease, but a pathological change. Cause corneal neovascularization for many reasons, immune inflammation, infectivity, degeneration, traumatic, improper use of contact lenses, iatrogenic diseases. Corneal neovascularization caused by the above reasons, if not treated in time, will eventually lead to corneal blindness. Corneal neovascularization is the leading cause of vision loss and blindness in the world, with 4.14% of ophthalmic patients having corneal neovascularization in the United States, 1.4 million newly-diagnosed corneal neovascularization patients each year in the United States, and 12% of them cause vision loss. In the United States, Approximately 170,000 new corneal neovascularization patients with decreased vision loss each year in the United States. There is no epidemiological investigation on corneal neovascularization in China. According to 4% of corneal neovascularization in the United States, 12% of them will result in loss of vision. In 2009, there were 62.83 million ophthalmic patients nationwide and about 300,000 New patients affected vision due to corneal neogenesis. Corneal neovascularization in patients with the progress will lead to further deterioration of visual acuity, eventually requiring corneal transplantation to restore vision. Therefore, such patients need effective anti-angiogenic therapy to prevent further loss of vision while avoiding corneal transplantation, and doctors have no effective treatment for corneal neovascularization.

Many features of biopharmaceuticals, especially recombinant proteins, are completely different from those of small molecule chemical molecules. The unstable biodegradation reaction is a multi-step reaction. Due to the limitations of the current analytical methods, these multi-step degradation processes that occur in high-level structures are also difficult to accurately measure and their stability, especially under routine conditions, presents a significant challenge. The eye is one of the most important organs of the human body; nearly 80% of the brain's information comes from the eyes. At the same time, due to its special physiological structure, the eye is a relatively weak tissue with very high requirements on viscosity and osmotic pressure. Therefore, for ophthalmic preparations, especially when there is trauma ophthalmic preparations used by demanding.

The biological effects of VEGF (vascular endothelial growth factor) are all mediated through its specific receptor VEGFR (vascular endothelial cell growth factor receptor), which leads to ligand-mediated dimerization, The dimerization of the receptor causes autophosphorylation and dephosphorylation of adjacent receptor subunits, triggering signal transduction. VEGFRs are tyrosine protein kinases and are classified by their function and structure as follows: fins-like tyrosine kinase-1 (VEGFR-1 / Flt-1), kinase- KDR), fins-like tyrosine kinase-4 (VEGFR-3 / Flt-4) and some low molecular weight VEGFRs (neuropilin-1). Flt-1 and KDR mainly distributed on vascular endothelial cells, while Flt-4 mainly distributed on lymphatic endothelial cells. Flt-1 and KDR, which bind to VEGF mainly, have higher binding affinity to VEGF than KDR. They all are glycosylated transmembrane receptors that directly participate in the signal transduction of VEGF into cells. The combination of Flt-1 and VEGF can promote the formation of vascular endothelial cells and regulate the permeability of blood vessels. Combined with VEGF, KDR can promote the proliferation and maturation of vascular endothelial cells. VEGF antagonists, in particular, a class of artificially engineered targeted engineered proteins such as Lucentis, Avastin, VEGF-trap and the like, which effectively block the signaling mediated by vascular endothelial growth factor (VEGF) Neovascularization and for the treatment of diseases caused by

neovascularization, such as tumors, eyes, etc. However, the above listed preparations of the above drugs are in the form of injections by the direct injection of the vitreous body or systemic administration in the form of preparations for the patients Say, its poor compliance, and there are many inconveniences. Therefore, the researchers began to study a well-compliant and convenient and effective pharmaceutical preparation containing VEGF antagonist. US7303748 discloses an eye drop containing VEGF-trap, and the preparation formula thereof is 39.4-103.06 mg / ml VEGF-trap, 5 mM phosphoric acid, 5 mM citric acid, 100 mM sodium chloride, 0.005% Tween-20. However, the concentration of the ophthalmic solution is relatively large. Because of the instability of biologic preparations, many current anti-angiogenic patients are not willing to be hospitalized. High concentrations of biologics can easily cause inconvenience in storage and use, meanwhile, Ocular dosing is a special route of administration, with respect to pH, osmotic pressure and local irritation, the addition of bacteriostats and antimicrobials, etc., which make the costly and limited effects of ocular surface anti-angiogenic drugs . Therefore, the present invention provides a method for the treatment of ocular surface diseases such as corneal neovascularization, neovascular glaucoma, pterygium, chronic conjunctivitis and the like which are low in concentration and inhibit the neovascularization, The demand has a significant meaning.

Content of the invention

One of the technical problems to be solved by the present invention is to provide an ophthalmic solution containing VEGF antagonist which is small in concentration but effective in curative effect.

In order to solve the above technical problem, the present invention provides the following technical solutions:

In one aspect, the present invention provides an ophthalmic solution containing a VEGF antagonist comprising 0.05-9.0 mg / ml VEGF antagonist; preferably 0.1-1.0 mg / ml VEGF antagonist; more preferably containing 0.5 mg / ml of a VEGF antagonist; wherein the VEGF antagonist is preferably a fusion protein comprising a fragment of FLT-1 and KDR.

The aforementioned VEGF antagonist is preferably a fusion protein having one of the following structures:

a.FP1: FLTd2-KDRd3-Fc, which is a fusion protein of a second immunoglobulin-like region of FLT-1 and a third immunoglobulin-like region of KDR with a human immunoglobulin Fc fragment;

b.FP2, a protein consisting of a first immunoglobulin-like region of KDR, a second immunoglobulin-like region of FLT-1, and a third immunoglobulin-like region of KDR fused to a human immunoglobulin Fc fragment: KDRd1 -FLTd2-KDRd3-Fc;

c. FP3, a protein fused to a human immunoglobulin Fc fragment from the second immunoglobulin-like region of FLT-1 and the 3-4 immunoglobulin-like region of KDR: FLTd2-KDRd3,4-Fc;

d.FP4, a protein consisting of a second immunoglobulin-like region of FLT-1, a third immunoglobulin-like region of KDR, and a fourth inumunoglobulin-like region of FLT-1 fused to a human immunoglobulin Fc fragment :

FLTd2-KDRd3-FLTd4-Fc;

e.FP5, a protein consisting of the second immunoglobulin-like region of FLT-1 and the third immunoglobulin-like region of KDR fused to the human immunoglobulin Fc fragment: FLTd2-KDRd3,4,5-Fc ;or

f.FP6, consisting of a second immunoglobulin-like region of FLT-1, a third immunoglobulin-like region of KDR and a 4-5 immunoglobulin-like region of FLT-1 fused to a human immunoglobulin Fc fragment Protein:

FLTd2-KDRd3-FLTd4,5-Fc;

g. FP7, a fusion protein of the second immunoglobulin-like region of FLT-1 and the 3-4 immunoglobulin-like region of KDR: FLTd2-KDRd3,4;

h8.FP8, a protein fused to the second immunoglobulin-like region of FLT-1 and the third immunoglobulin-like region of KDR: FLTd2-KDRd3.

The amino acids FLT-1D2, FLT-1D4, KDRD1, KDR D3 and KDR D4 of the above FLT-1 and KDR immunoglobulin-like regions are shown in Tables 1-5, the amino acid sequence of the FP3 protein is shown in Table 6, the amino acid sequence of the FP1 protein See Table 7 for sequence information. The amino acid sequence of the FP7 protein is shown in Table 8, and the amino acid sequence of the FP8 protein is shown in Table 9 of the Sequence Listing.

The ophthalmic solution provided by the present invention may further contain one or more of the following components:

(a) 5-100 mM buffer wherein the acid is selected from one or more of Tris-HCl, citric acid, phosphoric acid, sodium hydrogen phosphate, sodium dihydrogen phosphate, acetic acid, succinic acid,

(b) 5-500 mM of a basic amino acid selected from one or a combination of lysine, arginine, and histidine;

(c) 0.1-30% salt osmolyte modifier, wherein the sugar is selected from one or more of sucrose, trehalose, mannitol, glycerol, propylene glycol, sorbitol, the salt is selected from sodium chloride or other One or a combination of pharmaceutically acceptable salts; (d) 0.005-0.1% of one or more surfactants or co-solvents selected from the group consisting of polyethylene glycol, Tween 20, Tween 80, propylene glycol , Dimethylsulfoxide or one or more of other pharmaceutically acceptable surfactants;

The solution of one or more of the above components has a pH of 6 to 8.3.

The VEGF antagonist described in the above eye drops is most preferably the fusion protein of SEQ ID No: 6.

The present invention still further provides eye drops containing the following components, the specific components are as follows:

(a) 0.05-9 mg / ml of the fusion protein of SEQ ID No: 6;

(b) 5-250 mM citric acid;

(c) one or two of arginine or histidine of 5-500 mM;

(d) 4-30% sucrose or trehalose;

(e) 0.01-0.1% of a surfactant or cosolvent selected from one or two of polyethylene glycol or Tween 20;

(f) adjusting the pH to 7.5 to 8.3.

The present invention still further provides an eye drop containing the following components, the specific components are as follows:

(a) 0.1-1 mg / ml of the fusion protein of SEQ ID No: 6;

(b) 10-50 mM citric acid;

(c) one or both of 50-100 mM arginine or histidine;

(d) 5-20% sucrose;

(e) 0.01-0.1% of a surfactant or cosolvent selected from one or two of polyethylene glycol or Tween 20;

(f) adjusting the pH to 7.5 to 8.3.

The present invention still further provides an ophthalmic solution containing the following components, the specific components of which are as follows:

(a) 0.5 mg / ml of the fusion protein of SEQ ID No: 6;

(b) 50 mM citric acid;

(c) 250 mM arginine;

(d) 12.5% sucrose;

(e) 0.05% Tween-20;

(f) adjusting the pH to 7.5 to 8.3.

The invention also provides the use of the above-mentioned eye drops in the preparation of a medicament for treating ocular surface diseases caused by angiogenesis or growth; the ocular surface diseases are preferably neovascularization, corneal neovascularization, ocular neovascularization or Any of pterygium or its complications.

The technical contents disclosed in the Chinese patents ZL200510073595.4 and ZL200610066257.2 are taken as references of the present application; the technical content of the Chinese non-published patent CN 201010267503.7 is also referred to in the present application.

Compared with the prior art, the method has the advantages that: the content of the fusion protein in the eye drops is very low and the curative effect is accurate; and the production cost can be greatly saved on the basis of the high stability of the low-concentration eye drops.

detailed description

The following examples are only for further explanation of the present invention, and should not be taken as limiting the scope of the present invention.

Example 1 Concomitant ophthalmic treatment of corneal neovascularization induced by alkali burn

Drugs: chlortetracycline hydrochloride eye ointment, specifications lot number: 2.0g / support, 411002, valid until 2014.12, Chongqing Kerui Pharmaceutical Co., Ltd.; Lidocaine hydrochloride injection specifications lot: 5ml / support, 0.1g / support, Valid until 2012.04., Tianjin Jiaozuo Pharmaceutical Co., Ltd.

Reagents: sodium hydroxide (NaOH), specifications batches: 500g / bottle, 20091223, Chengdu Kelong Chemical Reagent Factory.

Test sample:

A, Compstax Eyedrops (prepared according to Example 3) 10mg / ml, colorless and transparent liquid, 1ml / support, batch number: FR1108001, stored at 2-8 ° C, was added dropwise to the surface of the eye;

B, Compaq Xp eyedrops (prepared according to Example 3) 9mg / ml, colorless and transparent liquid, 1ml / support, batch number: FR1108002, stored at 2-8 ° C, was added dropwise to the surface of the eye;

C, dexamethasone, colorless and transparent liquid, 1ml / stick, lot number: FR1108003, stored at 2 ~ 8 °C, when used in drops on the surface of the eye;

D, Compaq the Xipu eye drops (prepared according to Example 3) 0.1mg / ml, colorless and transparent liquid, 1ml / support, batch number: FR1108004, stored at 2-8 ° C, was added dropwise to the surface of the eye;

E. No concomitant buffer (prepared as in Example 16), colorless and transparent liquid, 1ml / cartridge, batch number: FR1108005, stored at 2-8 ° C and added dropwise to the surface of the eye during use;

F, Compstax Suppository drops (prepared according to Example 3) 5mg / ml, colorless and transparent liquid, 1ml / support, batch number: FR1108006, stored at 2-8 ° C, was added dropwise to the surface of the eye;

By Hong Hong Pharmaceutical Group Enterprise Technology Center of Sinopharm Research Institute preparation room to provide.

Experimental methods and results:

Forty-eight healthy New Zealand rabbits without healthy eyes were weighed and were anesthetized with 3% sodium pentobarbital (1 ml / kg) and local anesthetic lidocaine hydrochloride solution was administered to the eyes at a dose of 20 μl / 9mm diameter filter paper, soaked in 1mol / LNaOH solution for about 10s, the filter paper with tweezers placed on a dry filter paper to absorb excess NaOH solution, the filter paper soaked in NaOH rabbit eyes cornea in the middle 60s Remove, wash the bottle quickly rinse the cornea with about 20ml of saline, and give prophylactic antibiotics to prevent infection (chlortetracycline hydrochloride ointment), 3 times / 2 days.

Inmediately after modeling, burn was divided into A (Compaqeus ophthalmic solution (prepared as in Example 3) 10 mg / ml), B (Compaqtec ophthalmic solution 9 mg / ml prepared according to Example 3) (Dexamethasone), D (Compstaxi ophthalmic solution (prepared according to Example 3) 0.1 mg / ml), E (formulation buffered solution without convapor (prepared according to Example 16)) and F (Compactix Eyedrops (prepared according to Example 3) 5mg / ml) seven groups on the day of modeling 0 days from the first day, the group A ~ F administration of the corresponding drugs, the frequency of administration 6 times / Day, the dose of 50μl / eye, continuous administration of 10 days, another two New Zealand rabbits set as normal group. At the same time every day to observe the cornea NV growth status and eye inflammation.

On the 10th day of administration, animals were anesthetized with 3% sodium pentobarbital solution (1 ml / kg), and the local anesthetic lidocaine hydrochloride solution was administered to the eye surface at a dose of 20 μl / eye. Under the observation of rabbit corneal NV clock direction, while 10 times and 16 times under the microscope. Collecting Images Clocks were corrected in Photoshop CS, and the area of corneal neoplasms was treated with ImagePro Plus. The area formula was: $S = C / 12 \times 3.1416 \times [R2- (RL) 2]$ NV is the number of clocks occupied by the point in time of growth, R is the length in the picture from the edge where the cornea contacts the sclera to the center of the cornea, L is the length in the picture from the base where the cornea and the sclera contact the edge NV to the end NV of the NV in the cornea , Take the longest one of the blood vessels in each hour. Statistical analysis of all data using T test variance

data

that is the average, s is the standard deviation.

Through the common ocular surface observation, it was found that the corneal vascular network at each burn injury was obviously hyperemic on the first and second day of burns; on the third day after burns, there was NV growth on the corneal edge; and on the fifth day, convalescent eye drops 5mg / ml and The NV of the corneal surface of the preparation buffer solution containing Conpoxib was significantly increased, dexamethasone, Compstax ophthalmic solution 10 mg / ml, Compstax solution 9 mg / ml, and Compstax ophthalmic solution 0.1 The NV of mg / ml group was not obvious. On the 7th day, the NV of cornea on the cornea was obviously increased in the cornea of the burn group and the buffered solution group without compressible corpus luteum. , While dexamethasone, Compstax eye drops 10 mg / ml, Compstax eye drops 9 mg / ml, Compstax eye drops 5 mg / ml, and Compromix eye drops 0.1 mg / ml Group NV rarely grows to the burned corneal margin; on day 10, the burn group and the buffered solution group that does not contain Compstax have grown into the cornea of the burn with NV, while dexamethasone, Compaqepip 10 mg / ml, Compaq topp eye drops 9mg / ml, Compaq Xi Pu 5mg / ml and Compaq Xi Pu 0.1mg / ml group NV into the cornea burn less.

Corneal neovascularization area: the 10th day of corneal NV area data analysis results show that, compared with the alkali burn group and the preparation buffer solution group does not contain ciniphexin, dexamethasone, Compstax 10mg / ml, Compaq Ceftazidime 9mg / ml, Compaqepip 5mg / ml and Compaqepip 0.1mg / ml can significantly inhibit the growth of corneal NV and reduce the area of NV, with statistical significance (P {0.05), of which dexamethasone Compression with Compaqepip 0.1 mg / ml inhibited NV growth best (P {0.01). Although no buprenorph preparations buffer solution did not inhibit corneal neovascularization, and the NV area was greater than the burn group, there was no significant difference. The results are shown in Table 1.

Table 1 various drugs on alkali burns caused by corneal NV in rabbits

Note: negative control drug-free buffer, positive control for dexamethasone, compared with the burn group, * P (0.05, ** P (0.01; compared with the B group, # P (0.05, ## P (0.01 ; Compared with E group, ^ P (0.05, ^^ P (0.01.

Example 2 Effect of low dose concomitant ophthalmic solution on corneal NV growth induced by alkali burn

Drugs: chlortetracycline hydrochloride eye ointment, specifications lot number: 2.0g / support, 411002, valid until 2014.12, Chongqing Kerui Pharmaceutical Co., Ltd.; Lidocaine hydrochloride injection specifications lot: 5ml / support, 0.1g / support, Valid until 2012.04., Tianjin Jiaozuo Pharmaceutical Co., Ltd.

Reagents: sodium hydroxide (NaOH), specifications batches: 500g / bottle, 20091223, Chengdu Kelong Chemical Reagent Factory.

Test sample:

A, Compaq Xp Eye Drops (prepared according to Example 3) 0.5mg / ml, colorless and transparent liquid, 800μl / support, batch number: 20111001, stored at 2 ~ 8 °C,

B, Compstax ophthalmic solution (prepared according to Example 3) 0.1mg / ml, colorless and transparent liquid, 800μl / branch, batch number: 20111001, stored at 2-8 °C, when used in drops on the eye surface;

C, Formulation buffer solution without Compaqep, colorless and transparent liquid, 800μl / stick, batch number: 20111001, stored at 2-8 °C, when used, drops into the eye surface;

D, Compaq the Xipu eye drops (prepared according to Example 3) 0.01mg / ml, colorless and transparent liquid, 800μl / support, batch number: 20111001, stored at 2 ~ 8 °C, when used in drops on the eye surface;

E, dexamethasone, colorless and transparent liquid, 800μl / support, batch number: 20111001, stored at 2 ~ 8 °C, when used, drops into the eye surface;

F, Compstax ophthalmic solution (prepared according to Example 3) 0.05mg / ml, colorless and transparent liquid, 800μl / branch, batch number: 20111001, stored at 2-8 °C,

G, Compstax Suppository (prepared according to Example 3) 1mg / ml, colorless and transparent liquid, 800μl / support, batch number: 20111001, stored at 2 ~ 8 °C,

By Hong Hong Pharmaceutical Group Enterprise Technology Center of Sinopharm Research Institute preparation room to provide.

Experimental methods and results:

Tests were divided into A (Compaqeus ophthalmic solution (prepared according to Example 3) 0.5 mg / ml), B (Compsto-ophthalmic solution 0.1 mg / ml prepared according to Example 3), C Concomitant formulation buffer (prepared as in Example 16) buffer), D (Conpstox ophthalmic solution (prepared as in Example 3) 0.01 mg / ml), E (dexamethasone), F Concomitant ophthalmic solution (prepared according to Example 3) 0.05 mg / ml) and G (Compstax ophthalmic solution (prepared according to Example 3) 1 mg / ml).

The test method is the same as in Example 1.

The ocular surface showed that on the 1st and 2nd day of burns, corneal edge blood vessels in each group had obvious congestion and partial eyelid swelling. On the 3rd day after burn, there was a trend of NV growth on the corneal edge and eyelid redness subsided. On the 5th day, There was NV growth on the corneal surface, but some rabbit eyelids appeared red and swollen. On the 7th day, the NV of corneal surface of each group obviously grew obviously, while the cornea NV of dexamethasone group was less, but the eyelid swelling of rabbits More severe. On the 10th day, corneal NV in each group with concomitant cyprocon was obviously growing, some corneal neovascularization had grown into the burn site, while NV in dexamethasone group had fewer corneal burns and no swelling in the eyelid of rabbits Restoration, while the group containing concomitant echinoderm rabbit erythema.

Corneal neovascularization effects: Day 10 corneal NV area data analysis showed that dexamethasone and concomitant ophthalmic solution 0.5 mg / ml significantly inhibited compared with the formulation buffer solution without conpiroxepin Corneal NV growth, reduced NV area, with statistical significance (P (0.05). Compaq Xi Pu eye drops 1mg / ml, Compaq Xi Pu eye drops 0.1mg / ml also inhibit corneal NV growth. Compaq Xupu eye drops 0.01mg / ml did not inhibit corneal neovascularization growth, and the NV area is greater than the burn group, but no significant difference. See Table 2.

Table 2 various drugs on alkali burns caused by corneal NV in rabbits

Note: Buffer control, * P (0.05, ** P (0.01.

Example 3 Preparation of Compstisept (FP3 Fusion Protein) Eye Drops

prescription:

FP3 fusion protein 10 mg / ml, 9 mg / ml, 5 mg / ml, 1 mg / ml, 0.5 mg / ml, 0.1 mg / ml, 0.05 mg / ml or 0.01 mg / ml

Preparation Methods: Concentrated fluid exchange of FP3 fusion protein after thawing, in the C-level clean to clean Taiwan (sterile cabinet), according to aseptic method by adding sterile filter medium containing 55mM citrate, 12.5% sucrose, 250mM Arginine and 0.05% Tween 20 and filtered to adjust the FP3 fusion protein to 10 mg / ml, 9 mg / ml, 5 mg / ml, 1 mg / ml, 0.5 mg / ml, 0.1 mg / ml, / ml or 0.01mg / ml, pH 7.5 ~ 8.3, aseptically packed into containers containing eye drops, stored at 2-8 °C.

Example 4 Preparation of Compstisept (FP3 Fusion Protein) Eye Drops

Prescription: FP3 fusion protein 0.5 mg / ml

Preparation Methods: After the concentrated liquid exchange of FP3 fusion protein was thawed, in the C clean to clean Taiwan (sterile cabinet), according to aseptic method by adding sterile filter medium containing 10mM citric acid, 8.0% sucrose, 5mM Arginine and 0.05% Tween 20, filtered and the FP3 fusion protein was adjusted to 0.5 mg / ml, pH 7.5 to 8.3, aseptically dispensed into containers containing eye drops and stored at 2-8 °C.

Example 5 Preparation of Compstisept (FP3 fusion protein) eye drops

prescription:

Preparation Methods: After the concentrated liquid exchange of FP3 fusion protein was thawed, in the C clean to clean Taiwan (sterile cabinet), according to aseptic method by adding sterile filter containing 100mM citric acid, 20.0% sucrose, 250mM Arginine and 0.10% Tween 20. The FP3 fusion protein was filtered and adjusted to 1.0 mg / ml. The pH was 7.5 to 8.3. Aseptically dispensed into containers containing eye drops and stored at 2-8 ° C .

Example 6 Preparation of Compstisept (FP3 fusion protein) eye drops

prescription:

Preparation Methods: After the concentrated fluid exchange FP3 fusion protein was thawed in the C-level clean to clean Taiwan (sterile cabinet), according to aseptic method by adding sterile filter containing 5mM sodium dihydrogen phosphate, 10.0% seaweed Sugar, 100 mM arginine and 0.01% PEG400, filtered and the FP3 fusion protein was adjusted to 0.1 mg / ml, pH 7.5 to 8.3, aseptically dispensed into containers containing eye drops and stored in 2- 8 ° C.

Example 7 Preparation of Conpillip (FP3 fusion protein) eye drops

prescription:

Preparation Methods: After the concentrated fluid exchange FP3 fusion protein was thawed, in the C clean to clean station (sterile cabinet), according to aseptic method by adding sterile filter medium containing 5mM citric acid, 4.0% sucrose, 4.0 % Sodium chloride, 100 mM arginine, 100 mM histidine, 0.05% Tween 20, 0.05% PEG400, filtered, and the FP3 fusion protein was adjusted to 0.05 mg / ml and the pH was 7.5 to 8.3. Packed in containers containing eye drops, stored at 2-8 °C.

Example 8 Preparation of Conpstisept (FP3 fusion protein) eye drops

prescription:

Preparation Methods: After the concentrated liquid was changed FP3 fusion protein was thawed, in the C-level clean to clean Taiwan (sterile cabinet), according to aseptic method by adding sterile filter containing 10mM citric acid, 30.0% sucrose, 500mM Arginine, 0.1% Tween 20 in buffer, filtered and the FP3 fusion protein was adjusted to 5.0 mg / ml, pH 7.5 to 8.3, aseptically dispensed into containers containing eye drops and stored at 2-8 °C.

Example 9 Preparation of CompBip (FP3 fusion protein) eye drops

prescription:

Preparation Methods: After the concentrated liquid was changed into liquid FP3 fusion protein was thawed, in the C-level clean to clean Taiwan (sterile cabinet), according to aseptic method by adding sterile filter medium containing 10mM citric acid, 5.0% sucrose, 100mM Arginine, 0.05% Tween 20 buffer, filter, adjust FP3 fusion protein to 0.5mg / ml, pH 7.5 ~ 8.3, aseptically packed into containers containing eye drops, stored in 2-8 °C.

Example 10 Preparation of Compstisept (FP3 fusion protein) eye drops

prescription:

Preparation Methods: After the concentrated liquid exchange of FP3 fusion protein after thawing, in the C-level clean to clean Taiwan (sterile cabinet), according to aseptic method by adding sterile filter containing 250mM citric acid, 8.0% sucrose, 100mM Histidine, 0.10% Tween 20, filtered and the FP3 fusion protein was adjusted to 0.5 mg / ml, pH 7.5 to 8.3, sterile dispensed into containers containing eye drops and stored at 2-8 °C.

Example 13 Preparation of Compstisept (FP3 fusion protein) eye drops

prescription:

Preparation Methods: After the concentrated liquid was changed into liquid FP3 fusion protein was thawed, in the C-level clean to clean Taiwan (sterile cabinet), according to aseptic method by adding sterile filter medium containing 10mM citric acid, 5.0% sucrose, 100mM Arginine, 0.05% Tween 20 buffer, filter, adjust FP3 fusion protein to 0.5mg / ml, pH 7.5 ~ 8.3, aseptically packed into containers containing eye drops, stored in 2-8 °C.

Example 14 Preparation of a series of fusion protein eye drops

prescription:

Preparation Methods: The concentrated liquid after the conversion of a variety of fusion proteins (FP1, FP2, FP4, FP5, FP6, FP7, FP8) of the original liquid after thawing, in the C clean to clean station (sterile cabinet), according to aseptic operation The filter-sterilized buffer containing 55 mM citrate, 12.5% sucrose, 250 mM arginine and 0.05% Tween 20 was added and filtered to adjust the fusion protein to 9 mg / ml, 5 mg / ml, 1 mg / ml, / ml, 0.1mg / ml or 0.05mg / ml, pH 6.0 ~ 8.3, aseptically dispensed into containers containing eye drops, stored at 2-8 °C.

Example 15 Preparation of FP1 fusion protein eye drops

prescription:

Preparation Methods: The concentrated liquid CF1 fusion protein after thawing, in the C-level clean to clean Taiwan (sterile cabinet), according to aseptic method by adding sterile filter medium containing 5mM phosphate, 5mM citric acid, 100mM chlorine Sodium chloride and 0.005% Tween 20, filtered, and the fusion protein was adjusted to 1.0 mg / ml, pH 6.0-8.0, aseptically dispensed into containers containing eye drops and stored at 2-8 ° C.

Example 16 Preparation of a formulation buffer that does not contain conipustine (FP3 fusion protein)

prescription:

Preparation Methods: Aseptically added by filtration sterile bacteria containing 55mM citrate, 12.5% sucrose, 250mM arginine and 0.05% Tween 20 was filtered, respectively, the pH was adjusted to 7.5 to 8.3, sterile Dispense into containers containing eye drops and store at 2-8 ° C.

Example 17 Stability of 0.1 mg / ml CompBip (FP3 fusion protein) eye drops at 25 ° C

1、A 0.1 mg / ml Conberciph (FP3 fusion protein) eye drop was prepared as in Example 3

2、The above 0.1 mg / ml conbercip (FP3 fusion protein) eye drops, 25 ° C to stay like, in 0,1,2,3,4,5,6,7,8,9,10,11,12 Samples were taken monthly to determine the stability by SEC-HPLC. The results showed that the formulation can effectively inhibit the formation of polymers, product purity did not decline, the affinity of the fusion protein and VEGF almost unchanged. The specific results in Table 3.

Table 3. Stability of 0.1 mg / ml FP3 fusion protein at 25 ° C

Time (months) Appearance pH Polymer (%) Affinity (pM) 0 Pass 7.2 0.01 10.6 1 Pass 7.4 0.03 10.5 2 Pass 7.5 0.03 10.4 3 Pass 7.5 0.05 10.4 6 Pass 7.7 0.07 10.2 9 Pass 7.6 0.08 10.3 12 Pass 7.5 0.09 10.1

Example 18 Stability of 10 mg / ml CompBip (FP3 fusion protein) eye drops at 4 ° C

1、Concomitant treatment with 10 mg / ml of Conpituxel (FP3 fusion protein) as described in Example 3 was performed

2、The above 10 mg / ml conbercip (FP3 fusion protein) eye drops were retained at 4 ° C for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, Samples were assayed for stability by SEC-HPLC. The results showed that the formulation can effectively inhibit the formation of polymer, the product of the polymer increased slowly, the affinity of the fusion protein and VEGF almost unchanged. The specific results in Table 4.

Table 4. Stability of 10 mg / nd FP3 fusion protein at 4 ° C

SEQUENCE LISTING

(110) Chengdu Kang Hong Biotechnology Co., Ltd.

(120) An ophthalmic solution containing a VEGF antagonist

(130) ZL200610066257.2

(160) 9

(170) PatentIn version 3.3

(210) 1

(211) 93

(212) PRT

(213) FLT-1D2

(400) 1

Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr

1 5 10 15

Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile

20 25 30

Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly

35 40 45

Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala

50 55 60

Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly

65 70 75 80

His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr

85 90

(210) 2

(211) 96

(212) PRT

(213) FLT-1D4

(400) 2

Phe Ile Thr Val Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala

1 5 10 15

Gly Lys Arg Ser Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser

20 25 30

Pro Glu Val Val Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser

35 40 45

Ala Arg Tyr Leu Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr

50 55 60

Glu Glu Asp Ala Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser

65 70 75 80

Asn Val Phe Lys Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro

85 90 95

(210) 3

(211) 86

(212) PRT

(213) KDRD1

(400) 3

Pro Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr

1 5 10 15

Thr Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp

20 25 30

Pro Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys

35 40 45

Ser Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly

50 55 60

Asn Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala

65 70 75 80

Ser Val Ile Tyr Val Tyr

85

(210) 4

(211) 102

(212) PRT

(213) KDR D3

(400) 4

Val Val Leu Ser Pro Ser His Gly Ile Ile Glu Glu Leu Ser Val Gly

1 5 10 15

Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly

20 25 30

Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys

35 40 45

Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys

50 55 60

Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly

65 70 75 80

Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser

85 90 95

Thr Phe Val Arg Val His

100

(210) 5

(211) 92

(212) PRT

(213) KDR D4

(400) 5

Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu Val Glu Ala Thr Val

1 5 10 15

Gly Glu Arg Val Arg Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro Pro

20 25 30

Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr

35 40 45

Ile Lys Ala Gly His Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp

50 55 60

Thr Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro Ile Ser Lys Glu Lys

65 70 75 80

Gln Ser His Val Val Ser Leu Val Val Tyr Val Pro

85 90

(210) 6

(211) 552

(212) PRT

(213) artificial sequence

(400) 6

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser

1 5 10 15

Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu

20 25 30

Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu

35 40 45

Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu

50 55 60

Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile

65 70 75 80

Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu

85 90 95

Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys

100 105 110

Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val

115 120 125

Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val

130 135 140

Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn

145 150 155 160

Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg

165 170 175

Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr

180 185 190

Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys

195 200 205

Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg

210 215 220

Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu

225 230 235 240

Val Glu Ala Thr Val Gly Glu Arg Val Arg Leu Pro Ala Lys Tyr Leu

245 250 255

Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Leu

260 265 270

Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr Ile Met Glu

275 280 285

Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro

290 295 300

Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val Val Tyr Val

305 310 315 320

Pro Pro Gly Pro Gly Asp Lys Thr His Thr Cys Pro Leu Cys Pro Ala

325 330 335

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro

340 345 350

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val

355 360 365

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val

370 375 380

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln

385 390 395 400

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln

405 410 415

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala

420 425 430

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro

435 440 445

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr

450 455 460

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser

465 470 475 480

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr

485 490 495

Lys Ala Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr

500 505 510

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe

515 520 525

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys

530 535 540

Ser Leu Ser Leu Ser Pro Gly Lys

545 550

(210) 7

(211) 455

(212) PRT

(213) artificial sequence

(400) 7

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser

1 5 10 15

Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu

20 25 30

Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu

35 40 45

Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu

50 55 60

Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile

65 70 75 80

Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu

85 90 95

Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys

100 105 110

Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val

115 120 125

Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val

130 135 140

Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn

145 150 155 160

Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg

165 170 175

Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr

180 185 190

Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys

195 200 205

Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg

210 215 220

Val His Glu Lys Asp Lys Thr His Thr Cys Pro Leu Cys Pro Ala Pro

225 230 235 240

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys

245 250 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val

260 265 270

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp

275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr

290 295 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp

305 310 315 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu

325 330 335

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg

340 345 350

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys

355 360 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp

370 375 380

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys

385 390 395 400

Ala Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser

405 410 415

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser

420 425 430

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser

435 440 445

Leu Ser Leu Ser Pro Gly Lys

450 455

(210) 8

(211) 308

(212) PRT

(213) artificial sequence

(400) 8

Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His

1 5 10 15

Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro

20 25 30

Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro

35 40 45

Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser

50 55 60

Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val

65 70 75 80

Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn

85 90 95

Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser

100 105 110

Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn

115 120 125

Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His

130 135 140

Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met

145 150 155 160

Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp

165 170 175

Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys

180 185 190

Asn Ser Thr Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly

195 200 205

Ser Gly Met Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg

210 215 220

Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr

225 230 235 240

Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His

245 250 255

Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr

260 265 270

Val Ile Leu Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val

275 280 285

Ser Leu Val Val Tyr Val Pro Pro Asp Lys Thr His Thr Cys Pro Leu

290 295 300

Cys Pro Ala Pro

305

(210) 9

(211) 214

(212) PRT

(213) artificial sequence

(400) 9

Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His

1 5 10 15

Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro

20 25 30

Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro

35 40 45

Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser

50 55 60

Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val

65 70 75 80

Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn

85 90 95

Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser

100 105 110

Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn

115 120 125

Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His

130 135 140

Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met

145 150 155 160

Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp

165 170 175

Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys

180 185 190

Asn Ser Thr Phe Val Arg Val His Glu Lys Asp Lys Thr His Thr Cys

195 200 205

Pro Leu Cys Pro Ala Pro

210

CN107115294A VEGF antagonist-containing eye drops

1.An ophthalmic solution containing a VEGF antagonist, characterized by containing

(a) 0.05-9 mg / ml of the fusion protein of SEQ ID No: 6;

(b) 5-250 mM citric acid;

(c) one or two of arginine or histidine of 5-500 mM;

(d) 4-30% sucrose or trehalose;

(e) 0.01-0.1% of a surfactant or cosolvent selected from one or two of polyethylene glycol or Tween 20;

(f) adjusting the pH to 7.5 to 8.3.

2.The ophthalmic solution according to claim 1, which contains

(a) 0.5 mg / ml of the fusion protein of SEQ ID No: 6;

(b) 50 mM citric acid;

(c) 250 mM arginine;

(d) 12.5% sucrose;

(e) 0.05% Tween-20;

(f) adjusting the pH to 7.5 to 8.3.

3.The ophthalmic solution according to claim 1, which contains

(a) 0.1 mg / ml of the fusion protein of SEQ ID No: 6;

(b) 50 mM citric acid;

(c) 250 mM arginine;

(d) 12.5% sucrose;

(e) 0.05% Tween-20;

(f) adjusting the pH to 7.5 to 8.3.

4.The ophthalmic solution according to claim 1, which contains

(a) 1 mg / ml of the fusion protein of SEQ ID No: 6;

(b) 50 mM citric acid;

(c) 250 mM arginine;

(d) 12.5% sucrose;

(e) 0.05% Tween-20;

(f) adjusting the pH to 7.5 to 8.3.

5.Use of the ophthalmic solution according to any one of claims 1 to 4 in the manufacture of a medicament for the treatment of ocular surface diseases caused by angiogenesis or growth.

6.Use of the ophthalmic solution according to claim 5 for the manufacture of a medicament for the treatment of ocular surface diseases caused by angiogenesis or growth characterized in that the ophthalmological diseases are neovascularization, corneal neovascularization after corneal transplantation, Any of neovascularization or pterygium or its complications.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 107115294 A
(43)申请公布日 2017.09.01

(21)申请号 201710357021.2

(22)申请日 2012.01.19

(62)分案原申请数据
201210017896.5 2012.01.19

(71)申请人 成都康弘生物科技有限公司
地址 610036 四川省成都市金牛区蜀西路36号

(72)发明人 柯潇 郑强

(51)Int.Cl.
A61K 9/08(2006.01)
A61K 39/395(2006.01)
A61K 47/26(2006.01)
A61K 47/18(2006.01)
A61K 47/12(2006.01)
A61P 27/02(2006.01)

权利要求书1页 说明书12页
序列表8页

(54)发明名称

一种含有VEGF拮抗剂的滴眼液

(57)摘要

本发明涉及一种含有VEGF拮抗剂的滴眼液，该滴眼液中含有低浓度的VEGF拮抗剂，尤其是含有0.05-9.0mg/ml的VEGF拮抗剂，本发明滴眼液在确保安全稳定的基础上其疗效显著。

CN 107115294 A

1.一种含有VEGF拮抗剂的滴眼液,其特征在于含有

(a)0.05-9mg/ml的如SEQ ID No:6的融合蛋白;

(b)5-250mM的柠檬酸;

(c)5-500mM的精氨酸或组氨酸的一种或两种;

(d)4-30%的蔗糖或海藻糖;

(e)0.01-0.1%的表面活性剂或助溶剂,选自聚乙二醇或吐温20的一种或两种;

(f)调节pH为7.5～8.3。

2.根据权利要求1所述的滴眼液,其特征在于含有

(a)0.5mg/ml的如SEQ ID No:6的融合蛋白;

(b)50mM的柠檬酸;

(c)250mM的精氨酸;

(d)12.5%的蔗糖;

(e)0.05%的吐温-20;

(f)调节pH为7.5～8.3。

3.根据权利要求1所述的滴眼液,其特征在于含有

(a)0.1mg/ml的如SEQ ID No:6的融合蛋白;

(b)50mM的柠檬酸;

(c)250mM的精氨酸;

(d)12.5%的蔗糖;

(e)0.05%的吐温-20;

(f)调节pH为7.5～8.3。

4.根据权利要求1所述的滴眼液,其特征在于含有

(a)1mg/ml的如SEQ ID No:6的融合蛋白;

(b)50mM的柠檬酸;

(c)250mM的精氨酸;

(d)12.5%的蔗糖;

(e)0.05%的吐温-20;

(f)调节pH为7.5～8.3。

5.根据权利要求1-4中任一项所述的滴眼液在制备治疗由血管新生或生长引起的眼表疾病的药物中的用途。

6.根据权利要求5所述的滴眼液在制备治疗由血管新生或生长引起的眼表疾病的药物中的用途,其特征在于所述眼表疾病为角膜移植术后新生血管、角膜新生血管、眼表新生血管或翼状胬肉的任一种或其并发症。

一种含有VEGF拮抗剂的滴眼液

[0001] 本申请是申请号为CN201210017896.5，申请日为2012年1月19日，发明名称为“一种含有VEGF拮抗剂的滴眼液”的分案申请。

技术领域

[0002] 本发明涉及医药制剂领域，具体涉及一种含有VEGF拮抗剂的滴眼液。

背景技术

[0003] 角膜新生血管、新生血管性青光眼、翼状胬肉、慢性结膜炎等眼表病的发病均与新生血管的产生有一定的关系，VEGF的过度表达可以诱导眼表新生血管形成。其中角膜新生血管不是一种独立的角膜病，而是一种病理改变。导致角膜新生血管的原因很多，免疫炎症性、感染性、变性、外伤性、隐形眼镜的不当使用，医源性疾病等。上述原因导致的角膜新生血管如果不及时治疗，最终会导致角膜盲。角膜新生血管为世界范围视力丧失和失明的主要原因，在美国约4.14％的眼科患者患有角膜新生血管，美国每年有140万新增角膜新生血管患者，其中12％的会导致视力下降，在美国每年有约17万新增视力下降的角膜新生血管患者。中国尚没有关于角膜新生血管的流行病学调查，如果按照美国角膜新生血管占眼科患者4％，其中12％会导致视力下降来计算，2009年全国眼科患者为6283万，国内每年约有30万的新增患者因角膜新生而影响视力。患者角膜新生血管进展会导致视力的进一步恶化，最终需要角膜移植才能恢复视力。因此此类患者需要进行有效的抗新生血管治疗，以防止视力进一步丧失，同时避免进行角膜移植，医生对于角膜新生血管没有有效的治疗手段。

[0004] 生物药物，尤其是重组类蛋白许多特性完全不同于小分子化学分子，其发生不稳定降解反应是一个多步骤反应。限于目前分析方法的局限，发生于高级结构的这些饱含多个步骤的降解也很难精确测量到，其稳定性，尤其在常规条件下的保存就是一个很大的挑战。眼睛是人体最重要的器官之一：大脑的近80％的信息来自眼睛；同时眼睛由于其特殊的生理构造，是比较柔弱的组织，对粘度，渗透压等要求都非常高。因此对于眼用制剂，尤其是有外伤时候采用的眼用制剂要求异常严格。

[0005] VEGF(血管内皮细胞生长因子)的生物学效应均是通过其特异性受体VEGFR(血管内皮细胞生长因子受体)介导来实现的，VEGFR可导致由配体介导的二聚体化，受体的二聚体化促使相邻受体亚基自身磷酸化和去磷酸化，从而触发信号转导。VEGFR均为酪氨酸蛋白激酶，按其功能和结构分为：fins-样酪氨酸激酶-1(VEGFR-1/Flt-1)、激酶插入区受体(VEGFR-2/Flk-1/KDR)、fins-样酪氨酸激酶-4(VEGFR-3/Flt-4)及一些低相对分子质量VEGFR(neuropilin-1)。Flt-1和KDR主要分布于血管内皮细胞上，Flt-4则主要分布于淋巴管内皮细胞上。与VEGF相结合的主要为Flt-1和KDR，Flt-1与VEGF的结合力较KDR高，他们都是糖基化的跨膜受体，直接参与VEGF进入细胞内的信号传递。Flt-1与VEGF结合后能促使血管内皮细胞的形成和调节血管渗透性；KDR与VEGF结合后则能促进血管内皮细胞的增生及成熟。VEGF拮抗剂，尤其是一类人工设计的靶向基因工程蛋白，如Lucentis、Avastin、VEGF-trap等等，它们能有效地阻断由血管内皮生长因子(VEGF)介导的信号传递，抑制病变新生

血管的生长，并用于治疗肿瘤、眼睛等由新生血管引起的疾病，但是上述药物的已上市的制剂形式为注射剂，其给药方式为玻璃体直接注射或全身给药，其制剂形式对于患者而言，其依从性差，且存在诸多不便。因此，研究者开始研究一种患者依从性好，且方便有效的含有VEGF拮抗剂的药物制剂形式，US7303748公开了一种含有VEGF-trap的滴眼液，其制剂处方：39.4-103.06mg/ml VEGF-trap，5mM磷酸，5mM柠檬酸，100mM氯化钠，0.005％吐温-20。但是该滴眼液浓度较大，由于生物制剂一般都存在不稳定的问题，目前眼表的抗血管治疗患者很多都不愿住院治疗，高浓度的生物制剂很容易造成保存和使用的不便，同时眼表给药是一个特殊的给药途径，对pH、渗透压和局部刺激性，不能添加抑菌剂和抗菌剂等，上述问题使得用于眼表抗新生血管治疗药物的成本昂贵，效果有限。因此，提供一种浓度低，抑制新生血管疗效确切的滴眼液，并将其用于治疗角膜新生血管、新生血管性青光眼、翼状胬肉、慢性结膜炎等多种眼表疾病，对于满足临床需求具有显著的意义。

发明内容

[0006] 本发明需要解决技术问题之一是提供一种浓度小但是疗效确切的含有VEGF拮抗剂的滴眼液。

[0007] 为了解决上述技术问题，本发明提供了如下技术方案：

[0008] 本发明一方面提供了一种含有VEGF拮抗剂的滴眼液，该滴眼液中含有0.05-9.0mg/ml的VEGF拮抗剂；优选含有0.1-1.0mg/ml的VEGF拮抗剂；更优选含有0.5mg/ml的VEGF拮抗剂；其中所述VEGF拮抗剂优选为含有FLT-1和KDR的片断的融合蛋白。

[0009] 上述VEGF拮抗剂优选具有如下结构之一的融合蛋白：

[0010] a.FP1，由FLT-1的第2免疫球蛋白样区域和KDR的第3免疫球蛋白样区域与人免疫球蛋白Fc片段融合而成的蛋白：FLTd2-KDRd3-Fc；

[0011] b.FP2，由KDR的第1免疫球蛋白样区域，FLT-1的第2免疫球蛋白样区域和KDR的第3免疫球蛋白样区域与人免疫球蛋白Fc片段融合而成的蛋白：KDRd1-FLTd2-KDRd3-Fc；

[0012] c.FP3，由FLT-1的第2免疫球蛋白样区域和KDR的第3-4免疫球蛋白样区域与人免疫球蛋白Fc片段融合而成的蛋白：FLTd2-KDRd3,4-Fc；

[0013] d.FP4，由FLT-1的第2免疫球蛋白样区域，KDR的第3免疫球蛋白样区域和FLT-1的第4免疫球蛋白样区域与人免疫球蛋白Fc片段融合而成的蛋白：

[0014] FLTd2-KDRd3-FLTd4-Fc；

[0015] e.FP5，由FLT-1的第2免疫球蛋白样区域和KDR的第3-5免疫球蛋白样区域与人免疫球蛋白Fc片段融合而成的蛋白：FLTd2-KDRd3,4,5-Fc；或

[0016] f.FP6，由FLT-1的第2免疫球蛋白样区域，KDR的第3免疫球蛋白样区域和FLT-1的第4-5免疫球蛋白样区域与人免疫球蛋白Fc片段融合而成的蛋白：

[0017] FLTd2-KDRd3-FLTd4,5-Fc；

[0018] g.FP7，由FLT-1的第2免疫球蛋白样区域和KDR的第3-4免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3,4；

[0019] h.FP8，由FLT-1的第2免疫球蛋白样区域和KDR的第3免疫球蛋白样区域融合而成的蛋白：FLTd2-KDRd3。

[0020] 以上FLT-1和KDR免疫球蛋白样区域的氨基酸FLT-1D2、FLT-1D4、KDRD1、KDR D3、

KDR D4见序列表1-5,FP3蛋白的氨基酸序列见序列表6、FP1蛋白的氨基酸序列见序列表7,FP7蛋白的氨基酸序列见序列表8,FP8蛋白的氨基酸序列见序列表9。

[0021] 本发明提供的滴眼液中,还可以含有以下组分的一种或多种:

[0022] (a)5-100mM缓冲液,其中的酸选自Tris-HCl,柠檬酸,磷酸、磷酸氢钠,磷酸二氢钠,醋酸,丁二酸,盐酸中的一种或多种;

[0023] (b)5-500mM碱性氨基酸选自赖氨酸,精氨酸,和组氨酸中的一种或其组合;

[0024] (c)0.1-30%盐渗透压剂调节剂,其中的糖选自蔗糖,海藻糖,甘露醇,甘油,丙二醇,山梨酯醇中的一种或多种,盐选自氯化钠或其它药学上可以接受的盐中的一种或其组合;(d)0.005-0.1%的一种或多种表面活性剂或助溶剂,选自聚乙二醇,吐温20,吐温80,丙二醇,二甲基亚砜或其它药学上可以接受的表面活性剂中的一种或多种;

[0025] 上述组分之一或多种配成的溶液pH为6～8.3。

[0026] 上述滴眼液中所述的VEGF拮抗剂最优选为如SEQ ID No:6的融合蛋白。

[0027] 本发明还进一步提供了含有如下组分的滴眼液,具体组分如下:

[0028] (a)0.05-9mg/ml的如SEQ ID No:6的融合蛋白;

[0029] (b)5-250mM的柠檬酸;

[0030] (c)5-500mM的精氨酸或组氨酸的一种或两种;

[0031] (d)4-30%的蔗糖或海藻糖;

[0032] (e)0.01-0.1%的表面活性剂或助溶剂,选自聚乙二醇或吐温20的一种或两种;

[0033] (f)调节pH为7.5～8.3。

[0034] 本发明再进一步提供了含有如下组分的滴眼液,具体组分如下:

[0035] (a)0.1-1mg/ml的如SEQ ID No:6的融合蛋白;

[0036] (b)10-50mM的柠檬酸;

[0037] (c)50-100mM的精氨酸或组氨酸的一种或两种;

[0038] (d)5-20%的蔗糖;

[0039] (e)0.01-0.1%的表面活性剂或助溶剂,选自聚乙二醇或吐温20的一种或两种;

[0040] (f)调节pH为7.5～8.3。

[0041] 本发明更进一步提供了含有如下组分的滴眼液,具体组分如下:

[0042] (a)0.5mg/ml的如SEQ ID No:6的融合蛋白;

[0043] (b)50mM的柠檬酸;

[0044] (c)250mM的精氨酸;

[0045] (d)12.5%的蔗糖;

[0046] (e)0.05%的吐温-20;

[0047] (f)调节pH为7.5～8.3。

[0048] 本发明还提供了上述滴眼液在制备治疗由血管新生或生长引起的眼表疾病的药物中的用途;优选为眼表疾病为角膜移植术后新生血管、角膜新生血管、眼表新生血管或翼状胬肉的任一种或其并发症。

[0049] 中国专利ZL200510073595.4和ZL200610066257.2公开的技术内容作为本申请的参考;中国未公开专利CN 201010267503.7的技术内容也作为本申请的参考。

[0050] 本发明与现有技术相比其优势在于:滴眼液中融合蛋白的含量极低、且疗效确切,

在低浓度滴眼液较高的稳定性的基础上能够大幅度节约生产成本。

具体实施方式

[0051] 以下实施例仅作为对本发明的进一步解释，不能作为是对本发明保护范围的限制。

[0052] 实施例1、康柏西普滴眼液对碱烧伤致角膜新生血管研究

[0053] 药品：盐酸金霉素眼膏，规格批号：2.0g/支，411002，有效期至2014.12，重庆科瑞制药有限责任公司；盐酸利多卡因注射液，规格批号：5ml/支，0.1g/支，有效期至2012.04.，天津药物焦作有限公司。

[0054] 试剂：氢氧化钠(NaOH)，规格批号：500g/瓶，20091223，成都市科龙化工试剂厂。

[0055] 受试样品：

[0056] A，康柏西普滴眼液(按照实施例3制备)10mg/ml，无色透明液体，1ml/支，批号：FR1108001，保存于2～8℃，使用时滴加入眼睛表面；

[0057] B，康柏西普滴眼液(按照实施例3制备)9mg/ml，无色透明液体，1ml/支，批号：FR1108002，保存于2～8℃，使用时滴加入眼睛表面；

[0058] C，地塞米松，无色透明液体，1ml/支，批号：FR1108003，保存于2～8℃，使用时滴加入眼睛表面；

[0059] D，康柏西普滴眼液(按照实施例3制备)0.1mg/ml，无色透明液体，1ml/支，批号：FR1108004，保存于2～8℃，使用时滴加入眼睛表面；

[0060] E，不含康柏西普制剂缓冲液(buffer)(按照实施例16制备)，无色透明液体，1ml/支，批号：FR1108005，保存于2～8℃，使用时滴加入眼睛表面；

[0061] F，康柏西普滴眼液(按照实施例3制备)5mg/ml，无色透明液体，1ml/支，批号：FR1108006，保存于2～8℃，使用时滴加入眼睛表面；

[0062] 均由康弘药业集团企业技术中心中化药研究部制剂室提供。

[0063] 实验方法及结果：

[0064] 取健康无眼疾病新西兰兔48只，称其体重，用3％戊巴比妥钠麻醉(1ml/kg)，并于眼睛表面施予局麻药盐酸利多卡因液，剂量为20μl/眼；制备9mm直径滤纸片，浸泡于1mol/L NaOH溶液中约10s，用镊子将滤纸片放置于干燥的滤纸上吸去多余的NaOH溶液，将浸有NaOH的滤纸片置于家兔双眼眼角膜正中60s后取下，迅速取洗瓶用约20ml生理盐水冲洗角膜，并给予预防抗生素防止感染(盐酸金霉素眼膏)，3次/2天。

[0065] 造模后随即分为烧伤、A(康柏西普滴眼液(按照实施例3制备)10mg/ml)、B(康柏西普滴眼液(按照实施例3制备)9mg/ml)、C(地塞米松)、D(康柏西普滴眼液(按照实施例3制备)0.1mg/ml)、E(不含康柏西普的制剂缓冲溶液(按照实施例16制备))和F(康柏西普滴眼液(按照实施例3制备)5mg/ml)七个组，造模当天为0天，从第1天开始，A～F组给予相应药物，给药频率6次/天，给药剂量每次50μl/眼，连续给药10天，另取2只新西兰兔设为正常组。每天给药同时，观察眼角膜NV生长状态及眼部是否有炎症反应。

[0066] 于给药第10天，以3％戊巴比妥钠溶液麻醉动物(1ml/kg)，并于眼睛表面施予局麻药盐酸利多卡因液，剂量为20μl/眼，在裂隙灯10倍物镜下观察兔眼角膜NV钟点方向，同时在10倍和16倍物镜下照相。采集图像在Photoshop CS进行钟点数校正，角膜新生面积采用

ImagePro Plus处理;面积公式:$S=C/12\times3.1416\times[R^2-(R-L)^2]$,C表示在图片中角膜边缘从有NV到无NV生长时点所占的钟点数,R表示在图片中从角膜与巩膜接触的边缘到角膜中心的长度,L表示在图片中从角膜与巩膜接触边缘NV的根部到角膜中NV的末端NV长度,每个钟点中取最长的一根血管长度。所有数据统计分析采用T检验方差分析。数据以$\bar{x}\pm s$表示,是平均值,s是标准偏差。

[0067] 通过一般眼表观察发现,烧伤第1,2天各组烧伤眼角膜边缘血管网充血明显;烧伤第3天,角膜边缘有NV生长;第5天康柏西普滴眼液5mg/ml和不含康柏西普的制剂缓冲溶液眼角膜表面NV生长明显,地塞米松、康柏西普滴眼液10mg/ml、康柏西普滴眼液9mg/ml和康柏西普滴眼液0.1mg/ml组NV生长不明显;第7天各组眼角膜表面NV生长明显,烧伤组和不含康柏西普的制剂缓冲溶液组角膜NV向烧伤角膜生长,部分NV已生长到烧伤角膜边缘,而地塞米松、康柏西普滴眼液10mg/ml、康柏西普滴眼液9mg/ml、康柏西普滴眼液5mg/ml和康柏西普滴眼液0.1mg/ml组很少有NV生长到烧伤角膜边缘;第10天时烧伤组和不含康柏西普的制剂缓冲溶液组已有NV长入烧伤角膜内,而地塞米松、康柏西普10mg/ml、康柏西普滴眼液9mg/ml、康柏西普5mg/ml和康柏西普0.1mg/ml组NV长入烧伤角膜较少。

[0068] 角膜新生血管面积影响:第10天角膜NV面积数据分析结果可知,与碱烧伤组和不含康柏西普的制剂缓冲溶液组相比,地塞米松、康柏西普10mg/ml、康柏西普滴眼液9mg/ml、康柏西普5mg/ml和康柏西普0.1mg/ml能够明显抑制角膜NV生长,减少NV面积,具有统计学意义($P<0.05$),其中地塞米松与康柏西普0.1mg/ml抑制NV生长最佳($P<0.01$)。虽然不含康柏西普的制剂缓冲溶液无抑制角膜新生血管生长作用,且NV面积大于烧伤组,但无显著性差异。结果如表1。

[0069] 表1各药物对碱烧伤致兔眼角膜NV的影响($\bar{x}\pm s$)

[0070]

组别	眼睛数	药物剂量(mg/ml)	给药体积(μl)	NV面积(mm^2)
正常组	2	——	——	0
烧伤组	10	——	——	31.08±6.091
不含康柏西普的制剂buffer E	11	0	50	46.271±33.461
地塞米松C	11	0.25	50	15.367±9.698**##^^
康柏西普滴眼液A	11	10	50	20.16±13.829*#^
康柏西普滴眼液F	11	9	50	20.24±12.670*#^
康柏西普滴眼液B	10	5	50	20.618±14.073*#^
康柏西普滴眼液D	11	0.1	50	16.995±6.464**##^^

[0071] 注:阴性对照为不含药物的缓冲液,阳性对照为地塞米松,与烧伤组比较,*$P<0.05$,**$P<0.01$;与B组比较,#$P<0.05$,##$P<0.01$;与E组比较,^$P<0.05$,^^$P<0.01$。

[0072] 实施例2低剂量康柏西普滴眼液对碱烧伤致角膜NV生长影响

[0073] 药品:盐酸金霉素眼膏,规格批号:2.0g/支,411002,有效期至2014.12,重庆科瑞制药有限责任公司;盐酸利多卡因注射液,规格批号:5ml/支,0.1g/支,有效期至2012.04.,天津药物焦作有限公司。

[0074] 试剂:氢氧化钠(NaOH),规格批号:500g/瓶,20091223,成都市科龙化工试剂厂。

[0075] 受试样品:

[0076] A,康柏西普滴眼液(按照实施例3制备)0.5mg/ml,无色透明液体,800μl/支,批号:20111001,保存于2~8℃,使用时滴加入眼睛表面;

[0077] B,康柏西普滴眼液(按照实施例3制备)0.1mg/ml,无色透明液体,800μl/支,批号:20111001,保存于2~8℃,使用时滴加入眼睛表面;

[0078] C,不含康柏西普的制剂缓冲溶液,无色透明液体,800μl/支,批号:20111001,保存于2~8℃,使用时滴加入眼睛表面;

[0079] D,康柏西普滴眼液(按照实施例3制备)0.01mg/ml,无色透明液体,800μl/支,批号:20111001,保存于2~8℃,使用时滴加入眼睛表面;

[0080] E,地塞米松,无色透明液体,800μl/支,批号:20111001,保存于2~8℃,使用时滴加入眼睛表面;

[0081] F,康柏西普滴眼液(按照实施例3制备)0.05mg/ml,无色透明液体,800μl/支,批号:20111001,保存于2~8℃,使用时滴加入眼睛表面;

[0082] G,康柏西普滴眼液(按照实施例3制备)1mg/ml,无色透明液体,800μl/支,批号:20111001,保存于2~8℃,使用时滴加入眼睛表面;

[0083] 均由康弘药业集团企业技术中心中化药研究部制剂室提供。

[0084] 实验方法及结果:

[0085] 试验分为A(康柏西普滴眼液(按照实施例3制备)0.5mg/ml)、B(康柏西普滴眼液(按照实施例3制备)0.1mg/ml)、C(不含康柏西普的制剂缓冲液(按照实施例16制备)buffer)、D(康柏西普滴眼液(按照实施例3制备)0.01mg/ml)、E(地塞米松)、F(康柏西普滴眼液(按照实施例3制备)0.05mg/ml)和G(康柏西普滴眼液(按照实施例3制备)1mg/ml)八个组。

[0086] 试验方法操作同实施例1。

[0087] 通过眼表观察发现,烧伤第1,2天各组烧伤眼角膜边缘血管网充血明显,部分眼睑红肿现象;烧伤第3天,角膜边缘有NV生长趋势,眼睑红肿消退;第5天,各组角膜表面有NV生长,但是有部分兔眼睑又出现红肿现象;第7天各组眼角膜表面NV生长明显,有明显地向心性生长,而地塞米松组角膜NV较少,但兔眼睑红肿现象更加严重;第10天,含有康柏西普的各组角膜NV生长明显,部分角膜新生血管已长入烧伤部位,而地塞米松组NV长入角膜烧伤部位较少,兔眼睑红肿现象未见恢复,而含有康柏西普的各组未有兔眼睑红肿现象。

[0088] 角膜新生血管面积影响:第10天角膜NV面积数据分析结果可知,与不含康柏西普的制剂缓冲溶液组相比,地塞米松、康柏西普滴眼液0.5mg/ml能够明显抑制角膜NV生长,减少NV面积,具有统计学意义($P<0.05$)。康柏西普滴眼液1mg/ml、康柏西普滴眼液0.1mg/ml也能抑制角膜NV生长。而康柏西普滴眼液0.01mg/ml无抑制角膜新生血管生长作用,且NV面积大于烧伤组,但无显著性差异。见表2。

[0089] 表2各药物对碱烧伤致兔眼角膜NV的影响($\bar{x}\pm s$)

[0090]

组别	眼睛数	药物剂量（mg/ml）	给药体积(μl)	NV 面积(mm^2)	眼睛红肿数
正常组	1	------	------	0	
不含康柏西普的制剂缓冲液（buffer）C	10	0	50	35.16±17.614	1
阴性对照:地塞米松E	9	0.25	50	12.65±12.385**	4
康柏西普滴眼液G	12	1	50	24.56±15.498	1
康柏西普滴眼液A	12	0.5	50	20.10±9.643*	0
康柏西普滴眼液B	12	0.1	50	26.31±13.630	0
康柏西普滴眼液F	12	0.05	50	28.09±20.253	0
康柏西普滴眼液D	12	0.01	50	40.75±18.793	0

[0091] 注:Buffer对照,*P<0.05,**P<0.01。

[0092] 实施例3、康柏西普(FP3融合蛋白)滴眼液的制备

[0093] 处方:

[0094] FP3融合蛋白10mg/ml、9mg/ml、5mg/ml、1mg/ml、0.5mg/ml、0.1mg/ml、0.05mg/ml或0.01mg/ml

[0095]

柠檬酸	55 mM
蔗糖	12.5 %
精氨酸	250 mM
吐温 20	0.05 %

[0096] pH 7.5~8.3

[0097] 制备方法:将浓缩换液后的FP3融合蛋白原液化冻后,在C级洁净去洁净台(无菌柜)中,按无菌操作法加入过滤除菌的含有55mM柠檬酸、12.5%蔗糖、250mM精氨酸和0.05%吐温20的缓冲液、滤过、分别调节FP3融合蛋白至10mg/ml、9mg/ml、5mg/ml、1mg/ml、0.5mg/ml、0.1mg/ml、0.05mg/ml或0.01mg/ml,pH为7.5～8.3,无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0098] 实施例4、康柏西普(FP3融合蛋白)滴眼液的制备

[0099] 处方:FP3融合蛋白0.5mg/ml

[0100]

柠檬酸	10 mM
蔗糖	8.0 %
精氨酸	5 mM
吐温 20	0.05 %
pH	7.5~8.3

[0101] 制备方法:将浓缩换液后的FP3融合蛋白原液化冻后,在C级洁净去洁净台(无菌

柜)中,按无菌操作法加入过滤除菌的含有10mM柠檬酸、8.0%蔗糖、5mM精氨酸和0.05%吐温20的缓冲液,滤过、调节FP3融合蛋白至0.5mg/ml,pH为7.5～8.3,无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0102] 实施例5康柏西普(FP3融合蛋白)滴眼液的制备

[0103] 处方:

[0104]

FP3 融合蛋白	1.0 mg/ml
柠檬酸	100 mM
蔗糖	20.0 %
精氨酸	250 mM
吐温 20	0.10 %
pH	7.5~8.3

[0105] 制备方法:将浓缩换液后的FP3融合蛋白原液化冻后,在C级洁净去洁净台(无菌柜)中,按无菌操作法加入过滤除菌的含有100mM柠檬酸、20.0%蔗糖、250mM精氨酸和0.10%吐温20的缓冲液,滤过,调节FP3融合蛋白至1.0mg/ml,pH为7.5～8.3无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0106] 实施例6康柏西普(FP3融合蛋白)滴眼液的制备

[0107] 处方:

[0108]

FP3 融合蛋白	0.1mg/ml
磷酸二氢钠	5 mM
海藻糖	10.0 %
精氨酸	100 mM
PEG400	0.01 %
pH	7.5~8.3

[0109] 制备方法:将浓缩换液后的FP3融合蛋白原液化冻后,在C级洁净去洁净台(无菌柜)中,按无菌操作法加入过滤除菌的含有5mM磷酸二氢钠、10.0%海藻糖、100mM精氨酸和0.01%PEG400的缓冲液,滤过,调节FP3融合蛋白至0.1mg/ml,pH为7.5～8.3,无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0110] 实施例7康柏西普(FP3融合蛋白)滴眼液的制备

[0111] 处方:

FP3 融合蛋白	0.05 mg/ml
柠檬酸	5 mM
蔗糖	4.0 %
氯化钠	4.0%
精氨酸	100 mM
组氨酸	100 mM
吐温 20	0.05 %
PEG400	0.05%
pH	7.5~8.3

[0112]

[0113] 制备方法：将浓缩换液后的FP3融合蛋白原液化冻后，在C级洁净去洁净台（无菌柜）中，按无菌操作法加入过滤除菌的含有5mM柠檬酸、4.0％蔗糖、4.0％氯化钠、100mM精氨酸、100mM组氨酸、0.05％吐温20、0.05％PEG400的缓冲液，滤过，调节FP3融合蛋白至0.05mg/ml、pH为7.5～8.3，无菌分装到盛装滴眼液的容器中，存放于2-8℃。

[0114] 实施例8康柏西普（FP3融合蛋白）滴眼液的制备

[0115] 处方：

FP3 融合蛋白	5.0 mg/ml
柠檬酸	10 mM
蔗糖	30 %
精氨酸	500 mM
吐温 20	0.1 %
pH	7.5~8.3

[0116]

[0117]

[0118] 制备方法：将浓缩换液后的FP3融合蛋白原液化冻后，在C级洁净去洁净台（无菌柜）中，按无菌操作法加入过滤除菌的含有10mM柠檬酸、30.0％蔗糖、500mM精氨酸、0.1％吐温20的缓冲液，滤过，调节FP3融合蛋白至5.0mg/ml、pH为7.5～8.3，无菌分装到盛装滴眼液的容器中，存放于2-8℃。

[0119] 实施例9康柏西普（FP3融合蛋白）滴眼液的制备

[0120] 处方：

FP3 融合蛋白	0.5 mg/ml
柠檬酸	10 mM
蔗糖	5 %
精氨酸	100 mM
吐温 20	0.05 %
pH	7.5~8.3

[0121]

[0122] 制备方法：将浓缩换液后的FP3融合蛋白原液化冻后，在C级洁净去洁净台（无菌柜）中，按无菌操作法加入过滤除菌的含有10mM柠檬酸、5.0％蔗糖、100mM精氨酸、0.05％吐温20的缓冲液、滤过，调节FP3融合蛋白至0.5mg/ml、pH为7.5～8.3，无菌分装到盛装滴眼液的容器中，存放于2-8℃。

[0123] 实施例10康柏西普（FP3融合蛋白）滴眼液的制备

[0124] 处方：

[0125]

FP3 融合蛋白	0.5 mg/ml
柠檬酸	250 mM
蔗糖	8.0 %
组氨酸	100 mM
吐温 20	0.10 %
pH	7.5~8.3

[0126] 制备方法：将浓缩换液后的FP3融合蛋白原液化冻后，在C级洁净去洁净台（无菌柜）中，按无菌操作法加入过滤除菌的含有250mM柠檬酸、8.0％蔗糖、100mM组氨酸、0.10％吐温20的缓冲液、滤过，调节FP3融合蛋白至0.5mg/ml、pH为7.5～8.3，无菌分装到盛装滴眼液的容器中，存放于2-8℃。

[0127] 实施例13康柏西普（FP3融合蛋白）滴眼液的制备

[0128] 处方：

[0129]

FP3 融合蛋白	0.5 mg/ml
柠檬酸	10 mM
蔗糖	5 %
精氨酸	100 mM
吐温 20	0.05 %
pH	7.5~8.3

[0130] 制备方法：将浓缩换液后的FP3融合蛋白原液化冻后，在C级洁净去洁净台（无菌柜）中，按无菌操作法加入过滤除菌的含有10mM柠檬酸、5.0％蔗糖、100mM精氨酸、0.05％吐温20的缓冲液、滤过，调节FP3融合蛋白至0.5mg/ml、pH为7.5～8.3，无菌分装到盛装滴眼液的容器中，存放于2-8℃。

[0131] 实施例14系列融合蛋白滴眼液的制备

[0132] 处方：

[0133]

融合蛋白	9 mg/ml、5 mg/ml、1 mg/ml、0.5 mg/ml、0.1 mg/ml 或 0.05 mg/ml
柠檬酸	55 mM
蔗糖	12.5 %
精氨酸	250 mM
吐温 20	0.05 %
pH	7.5~8.3

[0134] 制备方法:将浓缩换液后的各种融合蛋白(FP1、FP2、FP4、FP5、FP6、FP7、FP8)原液化冻后,在C级洁净去洁净台(无菌柜)中,按无菌操作法加入过滤除菌的含有55mM柠檬酸、12.5%蔗糖、250mM精氨酸和0.05%吐温20的缓冲液、过滤,分别调节融合蛋白至9mg/ml、5mg/ml、1mg/ml、0.5mg/ml、0.1mg/ml或0.05mg/ml,pH为6.0~8.3,无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0135] 实施例15FP1融合蛋白滴眼液的制备

[0136] 处方:

[0137]

FP1 融合蛋白	1.0 mg/ml,
磷酸	5 mM
柠檬酸	5 mM
氯化钠	100 mM,
吐温-20	0.005%

[0138] pH 6.0

[0139] 制备方法:将浓缩换液后的FP1融合蛋白原液化冻后,在C级洁净去洁净台(无菌柜)中,按无菌操作法加入过滤除菌的含有5mM磷酸、5mM柠檬酸、100mM氯化钠和0.005%吐温20的缓冲液、滤过,调节融合蛋白至1.0mg/ml,pH为6.0-8.0,无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0140] 实施例16不含康柏西普(FP3融合蛋白)的制剂缓冲液制备

[0141] 处方:

[0142]

柠檬酸	55 mM
蔗糖	12.5 %
精氨酸	250 mM
吐温 20	0.05 %
pH	7.5~8.3

[0143] 制备方法:将按无菌操作法加入过滤除菌的含有55mM柠檬酸、12.5%蔗糖、250mM精氨酸和0.05%吐温20的缓冲液、滤过、分别调节pH为7.5~8.3,无菌分装到盛装滴眼液的容器中,存放于2-8℃。

[0144] 实施例17 0.1mg/ml康柏西普(FP3融合蛋白)滴眼液在25℃的稳定性

[0145] 1、按照实施例3制备0.1mg/ml康柏西普(FP3融合蛋白)滴眼液

[0146] 2、将上述0.1mg/ml康柏西普(FP3融合蛋白)滴眼液,25℃留样,在0,1,2,3,4,5,6,7,8,9,10,11,12月测定样品,通过SEC-HPLC确定稳定性。结果显示,该处方能有效抑制聚合物的生成,产品纯度基本不下降,融合蛋白与VEGF的亲和力几乎不变。具体结果见表3。

[0147] 表3. 0.1mg/ml FP3融合蛋白在25℃的稳定性

[0148]

时间(月)	外观	pH值	聚合物(%)	亲和力(pM)
0	合格	7.2	0.01	10.6
1	合格	7.4	0.03	10.5
2	合格	7.5	0.03	10.4
3	合格	7.5	0.05	10.4
6	合格	7.7	0.07	10.2
9	合格	7.6	0.08	10.3
12	合格	7.5	0.09	10.1

[0149] 实施例18 10mg/ml康柏西普(FP3融合蛋白)滴眼液在4℃的稳定性

[0150] 1、按照实施例3制备10mg/ml康柏西普(FP3融合蛋白)滴眼液

[0151] 2、将上述10mg/ml康柏西普(FP3融合蛋白)滴眼液,4℃留样,在0,1,2,3,4,5,6,7,8,9,10,11,12月测定样品,通过SEC-HPLC确定稳定性。结果显示,该处方能有效抑制聚合物的生成,产品中聚合物增加缓慢,融合蛋白与VEGF的亲和力几乎不变。具体结果见表4。

[0152] 表4. 10mg/ml FP3融合蛋白在4℃的稳定性

[0153]

时间（月）	外观	pH 值	浓度（mg/ml）	聚合物（%）	亲和力（pM）
0	合格	8.3	10.0	0.2	9.7
1	合格	8.3	10.1	0.2	9.6
2	合格	8.2	10.2	0.3	9.6
3	合格	7.9	10.0	0.4	9.3
6	合格	8.0	10.1	0.5	9.3
9	合格	7.9	10.1	0.6	9.1
12	合格	7.9	10.3	1.0	9.4

SEQUENCE LISTING

<110> 成都康弘生物科技有限公司

<120> 一种含有VEGF拮抗剂的滴眼液

<130> ZL200610066257.2

<160> 9

<170> PatentIn version 3.3

<210> 1

<211> 93

<212> PRT

<213> FLT-1D2

<400> 1

Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr
1 5 10 15

Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile
20 25 30

Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly
35 40 45

Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala
50 55 60

Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly
65 70 75 80

His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr
85 90

<210> 2

<211> 96

<212> PRT

<213> FLT-1D4

<400> 2

Phe Ile Thr Val Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala
1 5 10 15

Gly Lys Arg Ser Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser
20 25 30

Pro Glu Val Val Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser
35 40 45

Ala Arg Tyr Leu Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr
50 55 60

Glu Glu Asp Ala Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser
65 70 75 80

Asn Val Phe Lys Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro
85 90 95

<210> 3
<211> 86
<212> PRT
<213> KDRD1
<400> 3

Pro Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr
1 5 10 15
Thr Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp
20 25 30
Pro Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys
35 40 45
Ser Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly
50 55 60
Asn Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala
65 70 75 80
Ser Val Ile Tyr Val Tyr
85

<210> 4
<211> 102
<212> PRT
<213> KDR D3
<400> 4

Val Val Leu Ser Pro Ser His Gly Ile Ile Glu Glu Leu Ser Val Gly
1 5 10 15
Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly
20 25 30
Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys
35 40 45
Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys
50 55 60
Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly
65 70 75 80
Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser
85 90 95
Thr Phe Val Arg Val His
100

<210> 5

<211> 92
<212> PRT
<213> KDR D4
<400> 5

Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu Val Glu Ala Thr Val
1 5 10 15
Gly Glu Arg Val Arg Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro Pro
20 25 30
Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr
35 40 45
Ile Lys Ala Gly His Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp
50 55 60
Thr Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro Ile Ser Lys Glu Lys
65 70 75 80
Gln Ser His Val Val Ser Leu Val Val Tyr Val Pro
85 90

<210> 6
<211> 552
<212> PRT
<213> 人工序列
<400> 6

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
1 5 10 15
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu
20 25 30
Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu
35 40 45
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu
50 55 60
Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
65 70 75 80
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu
85 90 95
Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys
100 105 110
Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val
115 120 125
Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val
130 135 140

Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn
145 150 155 160
Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg
165 170 175
Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr
180 185 190
Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys
195 200 205
Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg
210 215 220
Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu
225 230 235 240
Val Glu Ala Thr Val Gly Glu Arg Val Arg Leu Pro Ala Lys Tyr Leu
245 250 255
Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Leu
260 265 270
Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr Ile Met Glu
275 280 285
Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro
290 295 300
Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val Val Tyr Val
305 310 315 320
Pro Pro Gly Pro Gly Asp Lys Thr His Thr Cys Pro Leu Cys Pro Ala
325 330 335
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
340 345 350
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
355 360 365
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
370 375 380
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
385 390 395 400
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
405 410 415
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
420 425 430
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
435 440 445
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr

450 455 460

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
465 470 475 480
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
485 490 495
Lys Ala Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
500 505 510
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
515 520 525
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
530 535 540
Ser Leu Ser Leu Ser Pro Gly Lys
545 550

<210> 7
<211> 455
<212> PRT
<213> 人工序列
<400> 7

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
1 5 10 15
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu
20 25 30
Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu
35 40 45
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu
50 55 60
Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
65 70 75 80
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu
85 90 95
Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys
100 105 110
Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val
115 120 125
Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val
130 135 140
Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn
145 150 155 160
Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg

165 170 175
Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr
180 185 190
Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys
195 200 205
Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg
210 215 220
Val His Glu Lys Asp Lys Thr His Thr Cys Pro Leu Cys Pro Ala Pro
225 230 235 240
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400
Ala Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445
Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 8

<211> 308

<212> PRT

<213> 人工序列

<400> 8

Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His
1 5 10 15
Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro
20 25 30
Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro
35 40 45
Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser
50 55 60
Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val
65 70 75 80
Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn
85 90 95
Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser
100 105 110
Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn
115 120 125
Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His
130 135 140
Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met
145 150 155 160
Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp
165 170 175
Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys
180 185 190
Asn Ser Thr Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly
195 200 205
Ser Gly Met Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg
210 215 220
Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr
225 230 235 240
Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His
245 250 255
Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr
260 265 270
Val Ile Leu Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val
275 280 285

Ser Leu Val Val Tyr Val Pro Pro Asp Lys Thr His Thr Cys Pro Leu
290 295 300
Cys Pro Ala Pro
305

<210> 9
<211> 214
<212> PRT
<213> 人工序列
<400> 9

Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His
1 5 10 15
Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro
20 25 30
Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro
35 40 45
Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser
50 55 60
Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val
65 70 75 80
Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn
85 90 95
Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser
100 105 110
Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn
115 120 125
Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His
130 135 140
Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met
145 150 155 160
Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp
165 170 175
Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys
180 185 190
Asn Ser Thr Phe Val Arg Val His Glu Lys Asp Lys Thr His Thr Cys
195 200 205
Pro Leu Cys Pro Ala Pro
210

Electronic Acknowledgement Receipt

EFS ID:	44366248
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	24-NOV-2021
Filing Date:	16-OCT-2020
Time Stamp:	14:39:27
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-008CIPCON6_2021-11-24_SuppIDS_Trans.pdf	50816 5facf57d151bd95ddd124b7b9479f7c0a14d7601	no	3

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	REGN-008CIPCON6_2021-11-24_SuppIDS_1449.pdf	76293 af8a62d6d29e4aa57c5f18815b9e41265192d92d	no	7
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Foreign Reference	F_16_CN1304427C_With-Machine-Translation.pdf	2341927 069bd3e3ea8adce34d48fd582387e4909483b0bd	no	39
Warnings:					
Information:					
4	Foreign Reference	F_17_CN100502945C.pdf	1545717 d64c716ef4fa34f5ab560ccd903c5c3a5b5d9f2f	no	19
Warnings:					
Information:					
5	Foreign Reference	F_18_CN100567325C_With-Machine-Translation.pdf	1928303 cf00465ced28d6f0bc1e6ccdcc33961de4971f6c	no	31
Warnings:					
Information:					
6	Foreign Reference	F_19_WO2012097019.pdf	5236088 2dab5a6035e196de265298bb14d42f765b32fc61	no	28
Warnings:					
Information:					
7	Foreign Reference	F_20_CN102233132B_With-Machine-Translation.pdf	1990774 e2a601c5028c677fb3b7e723ed3b95c83307490c	no	42
Warnings:					
Information:					
8	Foreign Reference	F_21_CN102380096B_With-Machine-Translation.pdf	1459040 6c371e2e6f71de06493c4738157d96416d0f27f8	no	28
Warnings:					
Information:					

9	Foreign Reference	F_22_CN103212075B_With-Machine-Translation.pdf	2933313 b4e30f035f9711866be09a8980d6c3e92ccfdd44	no	35
Warnings:					
Information:					
10	Foreign Reference	F_23_CN107115294A_With-Machine-Translation.pdf	2037855 a6b4121eadcfabe6117439e036694d62a7cec354	no	45
Warnings:					
Information:					
11	Non Patent Literature	NPL_24_Anonymous_MeetingArchive_PA003_EighteenMonth_Results_From_an_Extension_Study_2009.pdf	103027 9d04ea601630a08ef1e0d31547bcb4cdabee265f	no	1
Warnings:					
Information:					
12	Non Patent Literature	NPL_25_Anonymous_MeetingArchive_PA040_One_Year_Results_of_the_DA_VINCI_Study_2011.pdf	106035 23e17828500e03d3eae2f06226c07f3d0544fa4b	no	1
Warnings:					
Information:					
13	Non Patent Literature	NPL_26_Anonymous_MeetingArchive_PA080_One_Year_Results_2008.pdf	103644 ec0ce205eec0d059d11bc9b715cdbedf220af6fe	no	1
Warnings:					
Information:					
14	Non Patent Literature	NPL_27_Anonymous_MeetingArchive_PO259_OCT_and_Fluorescein_Angiography_Outcomes_2009.pdf	99987 77942be6456c1c00d71a80e3bdb812549cecf656	no	1
Warnings:					
Information:					
15	Non Patent Literature	NPL_28_Anonymous_MeetingArchive_PO260_VEGF_TrapEye_VisionSpecific_Quality_of_Life_2009.pdf	104493 e998f4d7d8e95bab67dae12893d744b0288212e4	no	1
Warnings:					
Information:					

16	Non Patent Literature	NPL_29_Anonymous_MeetingArchive_PO492_One_Year_Results_of_the_VIEW_1_and_VIEW_2_Studies_2011.pdf	105506 abf2285cf3a6ca1e0c77b786965a2abb88698444	no	1
Warnings:					
Information:					
17	Non Patent Literature	NPL_30_Anonymous_MeetingArchive_PO549_2011.pdf	101288 46a3d07139e52774c06185a2951761f5021ba848	no	1
Warnings:					
Information:					
18	Non Patent Literature	NPL_31_Anonymous_MeetingArchive_PO571_OCT_and_FluoresceinAngiographicOutcomes_2008.pdf	104280 7b13dd9e5948ebceac67cab62f75d71a44e3f3f5	no	1
Warnings:					
Information:					
19	Non Patent Literature	NPL_32_Bontempo_1997.pdf	2607980 6b4cafbef4d5772d91ed8825ea2a88f2bf1852f3	no	20
Warnings:					
Information:					
20	Non Patent Literature	NPL_33_Bressler_2001_Quality_Greyscale.pdf	598579 f6a28ec5240cee1aee0769704b9f016b32b430e8	no	10
Warnings:					
Information:					
21	Non Patent Literature	NPL_34_Brown_2011_Ranibizumab_for_Diabetic_Macular_Edema.pdf	82815 faa86eef3caa509c04096b59ac2d768d50e90bab	no	2
Warnings:					
Information:					
22	Non Patent Literature	NPL_35_Brown_2011_Sustained_Benefits.pdf	2221959 ab88edc2150994d2dd977ba0523a354d9c58a814	no	9
Warnings:					
Information:					

23	Non Patent Literature	NPL_36_Cao_2009.pdf	81340 8b1411b836fbe6adbe1d526a2ed90b7152ab3618	no	2
Warnings:					
Information:					
24	Non Patent Literature	NPL_37_CDE_21-756_2004.pdf	6245773 1a112e20830954cb65e674d11f2fdaf104baaacb	no	98
Warnings:					
Information:					
25	Non Patent Literature	NPL_38_CDE_125156_2006.pdf	13801393 281c5ec5b2d147d4e1450b1b3a74f1032932192c	no	171
Warnings:					
Information:					
26	Non Patent Literature	NPL_39_Cheung_2011.pdf	81192 422cad1ddd639d4b5ef99d75f79c2602efe7ddb5	no	2
Warnings:					
Information:					
27	Non Patent Literature	NPL_40_Dixon_2009.pdf	337202 19b764bea73706ac2eed58940cb312414325973f	no	8
Warnings:					
Information:					
28	Non Patent Literature	NPL_41_Eyetech_2003_Greyscale.pdf	859536 67f2703422d0a054da45f31357fbad960ada7c71	no	8
Warnings:					
Information:					
29	Non Patent Literature	NPL_42_Heier_2011_Ranibixumab_for_Choroidal_Neovascularization.pdf	1244849 6da6fd6fd5c36357dea7307514f3f816da1f49d5	no	8
Warnings:					
Information:					

30	Non Patent Literature	NPL_43_Heier_2016_Intravitreal_Aflibercept_for_DME_Greyscale.pdf	1013155 c09146259e22a50e9e5d2aa31a4bab89125d6cfd	no	10
Warnings:					
Information:					
31	Non Patent Literature	NPL_44_HERCEPTIN_label.pdf	198224 445889262cae30b449cf0cf9ea8fcefb80b60ad4	no	2
Warnings:					
Information:					
32	Non Patent Literature	NPL_45_NCT00637377_v1.pdf	1562090 ea466766ac44b747ab8e7ec57c9ebe8a52e773f7	no	20
Warnings:					
Information:					
33	Non Patent Literature	NPL_46_Ip_2009_Greyscale.pdf	10748590 b8eb81d9960966ce37a81d0cd37ea729df1025bf	no	16
Warnings:					
Information:					
34	Non Patent Literature	NPL_47_Kaiser_2009.pdf	80774 ce065bb2eb3b5d89bf1ef6bd3df58cf1384c90d5	no	2
Warnings:					
Information:					
35	Non Patent Literature	NPL_48_Korobelnik_2014.pdf	477357 043e442187d840dd14d2f5cb64181460028 18052	no	7
Warnings:					
Information:					
36	Non Patent Literature	NPL_49_Krzystolik_2002_Greyscale.pdf	1428664 6d87330b16028a7ccc4a10fa5fd4c02189873413	no	9
Warnings:					
Information:					

37	Non Patent Literature	NPL_50_Lalwani_2007_Split_Greyscale.pdf	1952936 6d8042b6697fbea5a2bb3446f32d61d18f8070c9	no	7
Warnings:					
Information:					
38	Non Patent Literature	NPL_51_Lobov_2011.pdf	74505 38f1b83b5521663405a48ff3ff1a1180c26a0add	no	2
Warnings:					
Information:					
39	Non Patent Literature	NPL_52_Lucentis_Approval_2006.pdf	739647 8e642fa4b11846e94d6abdfda772527c9290cb2e	no	6
Warnings:					
Information:					
40	Non Patent Literature	NPL_53_Lucentis_Label_2010.pdf	16678413 3287e10538ea753ebe4ec69715bd73a91fe96b0e	no	7
Warnings:					
Information:					
41	Non Patent Literature	NPL_54_Macular_1991.pdf	7281232 89606ff320111f007f39dbf86a821a3a1dd25ccd	no	12
Warnings:					
Information:					
42	Non Patent Literature	NPL_55_Mitchell_2009_Greyscale.pdf	15387484 a9d1aee5955a553a5de0d294eee62aa96a6abb2c	no	15
Warnings:					
Information:					
43	Non Patent Literature	NPL_56_Mitra_2011.pdf	925833 a1884f6952e581cfd2af4ce81170a739fcf4b8fe	no	7
Warnings:					
Information:					

44	Non Patent Literature	NPL_57_Mousa_2010.pdf	195361 ea7a6dc526337b906480ecf4b82412f6f563457e	no	13
Warnings:					
Information:					
45	Non Patent Literature	NPL_58_Nguyen_2006_A_Phase_I_Greyscale.pdf	2830227 830186121a084f242397b140029e4b21cb75dd82	no	14
Warnings:					
Information:					
46	Non Patent Literature	NPL_59_Regeneron_PR_2007-08-13_Greyscale.pdf	40759 c4255b39a18f84199c9072daa288f76e0bfc88a2	no	2
Warnings:					
Information:					
47	Non Patent Literature	NPL_60_Regeneron_An_Exploratory_ARVO_2007_Letter_Greyscale.pdf	242242 1e69e0593d87bc32fb32deb6ccf5638b56cb4b77	no	1
Warnings:					
Information:					
48	Non Patent Literature	NPL_61_Regeneron_CLEAR-IT-2_ARVO_2007_Letter_Greyscale.pdf	264645 ca0daaa1dd77b3756614fbfc8a26fa8afddd0d9a	no	1
Warnings:					
Information:					
49	Non Patent Literature	NPL_62_Regeneron_Optical_Coherence_ARVO_2007_Letter_Greyscale.pdf	193643 51adb576d0d9fc7edc737fb93c1556594185dfa5	no	1
Warnings:					
Information:					
50	Non Patent Literature	NPL_63_Regeneron_PR_2008-05-01_Greyscale.pdf	2231312 cd68ad965d7e488a4de1aeb4171171a2a54af1ac	no	6
Warnings:					
Information:					

51	Non Patent Literature	NPL_64_Form_10-Q_November_7_2007.pdf	432990 459649905a3e4f9ca0ce29e440c9986a9e1e5a47	no	69
Warnings:					
Information:					
52	Non Patent Literature	NPL_65_Regillo_2008.pdf	887640 1ddcfa88202f9e76b64f4d4b7a5ef7bbe15f93f4	no	15
Warnings:					
Information:					
53	Non Patent Literature	NPL_66_Rosenfeld_2005_Greyscale.pdf	7394769 26ca9f632b710ce297b9197a9d0492db9e8272f5	no	5
Warnings:					
Information:					
54	Non Patent Literature	NPL_67_Scott_2009_Greyscale.pdf	9210779 0e47a82afae924ed1f373ab39175c529f49c7950	no	15
Warnings:					
Information:					
55	Non Patent Literature	NPL_68_Simo_2009.pdf	112662 ba2fdcee8e3da7bae13e52defabe97f4136acff4	no	7
Warnings:					
Information:					
56	Non Patent Literature	NPL_69_Slides_for_2008_Retina_Society_Meeting_Presentation_Greyscale.pdf	1076125 0dae724f29405e69a3641f7998e8596f88137df6	no	29
Warnings:					
Information:					
57	Non Patent Literature	NPL_70_Tolentino_2011.pdf	73254 c55a90e491c59f6290110c4d26acd4d89cafa4a4	no	2
Warnings:					
Information:					

58	Non Patent Literature	NPL_71_vanBruggen_1999.pdf	289087 7c2066d68cb0ed94be5efe3c5dc2e77e45fe9b0f	no	9
Warnings:					
Information:					
59	Non Patent Literature	NPL_72_WHO_2006.pdf	165034 c1dd51ea7600f21dd41b685b0f1e9ea9e166da99	no	5
Warnings:					
Information:					
60	Non Patent Literature	NPL_73_IPR2021-00880_Institution_Decision.pdf	1663675 0f2fc6ce67f82f759ea6b91d5045a94c7cd9282e	no	51
Warnings:					
Information:					
			Total Files Size (in bytes):	134444112	

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

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT		
	Attorney Docket No.	REGN-008CIPCON6
	Confirmation No.	7325
	First Named Inventor	George D. Yancopoulos
	Application Number	17/072,417
	Filing Date	October 16, 2020
	Group Art Unit	To Be Assigned
Address to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Examiner Name	To Be Assigned
	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

The attention of the Examiner is invited to the documents listed on the attached Substitute 1449.

Copies of the U.S. patents and published applications listed on the attached Substitute 1449 are not submitted herewith, in accordance with the Strategic Plan Final Rule, 69 Fed. Reg. 56481-56547 (September 21, 2004), effective October 21, 2004.

Copies of the foreign publications and non-patent literature documents listed on the attached Substitute 1449 are submitted herewith. Applicant respectfully submits that a subset of references submitted herein were previously submitted in this or a priority application. Nonetheless, Applicant is submitting these previously submitted references to provide an accurate reference citation or to provide a clearer copy of the reference.

Applicant would also like to bring to the Examiner's attention that the PTAB has instated *inter partes* reviews for related U.S. Patent Nos. 9,254,338 and 9,669,069.

It is respectfully requested that the information above be expressly considered during the prosecution of this application, and that the documents be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

No aspect of these submissions constitute admission of prior art status or a disclaimer of claim scope.

Statements

☒ **No statement.** Because this Information Disclosure Statement is being submitted prior to issuance of the first action on the merits of the above-captioned application, no certification or fee is required.

☐ **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-008CIPCON6.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 24 November 2021

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
Reg. No. 28,807

BOZICEVIC, FIELD & FRANCIS LLP
201 Redwood Shores Parkway, Suite 200
Redwood City, CA 94065
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	17/072,417
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	1	of	1	Attorney Docket Number	REGN-008CIPCON6

U.S. PATENT DOCUMENTS					
Examiner Initial*	Cite No.	Patent Number Number-Kind Code (*if known*)	Issue Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	6897294	2005-05-24	Davis-Smyth et al.	

U.S. PATENT APPLICATION PUBLICATIONS					
Examiner Initial*	Cite No.	Publication Number **Number-Kind Code** (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1				

FOREIGN PATENT DOCUMENTS						
Examiner Initial*	Cite No.	Foreign Document Number Country Code-Number-Kind Code (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	1					

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

Examiner Signature		Date Considered	

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

Electronic Acknowledgement Receipt

EFS ID:	44539687
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	16-DEC-2021
Filing Date:	16-OCT-2020
Time Stamp:	17:02:36
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	REGN-008CIPCON6_2021-12-16_SuppIDS_Trans.pdf	51450 64ba6703728013555cfcf4fce803dd9776ec7463	no	2

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	REGN-008CIPCON6_2021-12-16_SuppIDS_SB08A.pdf	22114 7b19497ec9a8214ea8bf9165d66f764118c37018	no	1

Warnings:
Information:
This is not an USPTO supplied IDS fillable form

Total Files Size (in bytes):	73564

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

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT	Attorney Docket No.	REGN-008CIPCON6
	Confirmation No.	7325
	First Named Inventor	George D. Yancopoulos
	Application Number	17/072,417
	Filing Date	October 16, 2020
	Group Art Unit	To Be Assigned
	Examiner Name	To Be Assigned
Address to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

Applicant submits 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.

The publications discussed herein are provided to comply with the duty to disclose in accordance with 37 C.F.R. § 1.56. However, nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed

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 documents 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-008CIPCON6.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 16 December 2021

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
Reg. No. 28,807

BOZICEVIC, FIELD & FRANCIS LLP
201 Redwood Shores Parkway, Suite 200
Redwood City, CA 94065
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

Electronically Filed

PRELIMINARY AMENDMENT Under CFR 1.115 Address to: Mail Stop Patent Application Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450		
	Attorney Docket No.	REGN-008CIPCON6
	Confirmation No.	7325
	First Named Inventor	YANCOPOULOS, GEORGE D.
	Application Number	17/072,417
	Filing Date	October 16, 2020
	Group Art Unit	To Be Assigned
	Examiner Name	To Be Assigned
	Title: *"Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders"*	

Sir:

Prior to the examination of the above-referenced application on the merits, please enter the amendments below.

AMENDMENTS TO THE CLAIMS

1. - 101. (**Canceled**)

102. (New) A method for treating an angiogenic eye disorder in a patient in need thereof, said method comprising administering, by intravitreal injection, one or more maintenance doses of 2 mg aflibercept at least 8 weeks after the immediately preceding dose;

wherein said patient has previously received, by intravitreal injection, a single initial dose of 2 mg aflibercept, followed by one or more secondary doses of 2 mg aflibercept;

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

wherein the patient achieves a gain in visual acuity of at least 7 letters, compared to baseline by week 52, according to the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart.

103. (New) The method of claim 102, wherein the angiogenic eye disorder is age related macular degeneration.

104. (New) The method of claim 102, wherein the angiogenic eye disorder is diabetic retinopathy.

105. (New) The method of claim 102, wherein the angiogenic eye disorder is diabetic macular edema.

106. (New) The method of claim 102, wherein the angiogenic eye disorder is macular edema following retinal vein occlusion.

107. (New) The method of claim 102, wherein the method comprises administering, by intravitreal injection, two or more maintenance doses of 2 mg aflibercept, wherein each maintenance dose is administered at least 8 weeks after the immediately preceding dose.

108. (New) The method of claim 102, wherein the method comprises administering, by intravitreal injection, five or more maintenance doses of 2 mg aflibercept, wherein each maintenance dose is administered at least 8 weeks after the immediately preceding dose.

109. (New) The method of claim 108, wherein the patient achieves the gain in visual acuity by 24 weeks following the single initial dose.

110. (New) The method of claim 102, wherein the patient achieves a gain in visual acuity of at least 8 letters, compared to baseline, according to the ETDRS visual acuity chart.

111. (New) A method for treating macular edema following retinal vein occlusion in a patient in need thereof comprising administering to the patient, by intravitreal injection, a single initial dose of 2 mg aflibercept; followed by one or more doses of 2 mg aflibercept once every 4 weeks thereafter, and wherein the patient achieves a gain in visual acuity of at least 5 letters, compared to baseline, according to the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart.

112. (New) The method of claim 111, wherein the patient achieves the gain by 24 weeks following the single initial dose.

113. (New) The method of claim 112, wherein the patient achieves a gain of at least 10 letters, compared to baseline by week 24, according to the ETDRS visual acuity chart.

114. (New) The method of claim 112, wherein the patient achieves a gain of at least 15 letters, compared to baseline by week 24, according to the ETDRS visual acuity chart.

115. (New) The method of claim 111, wherein the patient achieves the gain by 52 weeks following the single initial dose.

116. (New) The method of claim 115, wherein the patient achieves a gain of at least 10 letters, compared to baseline by week 52, according to the ETDRS visual acuity chart.

117. (New) The method of claim 111, wherein the patient achieves a gain of at least 15 letters, compared to baseline by week 52, according to the ETDRS visual acuity chart.

118. (New) The method of claim 111, wherein five doses following the single initial dose are administered.

119. (New) A method for treating macular edema following retinal vein occlusion in a patient in need thereof comprising administering to the patient, by intravitreal injection, a single initial dose of 2 mg aflibercept followed by one or more doses of 2 mg aflibercept once every 4 weeks thereafter; and wherein the patient achieves a reduction in central retinal thickness of at least 400 micrometers as measured by optical coherence tomography compared to baseline.

120. (New) The method of claim 119, wherein the patient achieves the reduction in central retinal thickness by 24 weeks following the single initial dose.

121. (New) The method of claim 119, wherein the patient achieves the reduction in central retinal thickness by 52 weeks following the single initial dose.

122. (New) The method of claim 119, wherein five secondary doses are administered following the single initial dose.

123. (New) A method for treating macular edema following retinal vein occlusion in patients in need thereof comprising sequentially administering to each of said patients, by intravitreal injection, a single initial dose of 2 mg aflibercept, followed by one or more secondary doses of 2 mg of aflibercept, followed by one or more tertiary doses of 2 mg of aflibercept;

wherein each secondary dose is administered at least 4 weeks following the immediately preceding dose;

wherein each tertiary dose is administered at least 8 weeks following the immediately preceding dose; and

wherein at least 55 percent of said patients achieve an average gain in visual acuity of at least 5 letters, compared to baseline, according to the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart.

124. (New) The method of claim 123, wherein at least 55 percent of the patients achieve the gain by 24 weeks following the single initial dose.

125. (New) The method of claim 124, wherein at least 55 percent of the patients achieve a gain of at least 10 letters, compared to baseline by week 24, according to the ETDRS visual acuity chart.

126. (New) The method of claim 124, wherein at least 55 percent of the patients achieve a gain of at least 15 letters, compared to baseline by week 24, according to the ETDRS visual acuity chart.

127. (New) The method of claim 123, wherein at least 55 percent of the patients achieve the gain by 52 weeks following the initial dose.

128. (New) The method of claim 127, wherein at least 55 percent of the patients achieve a gain of at least 10 letters, compared to baseline by week 52, according to ETDRS visual acuity chart.

129. (New) The method of claim 127, wherein at least 55 percent of the patients achieve a gain of at least 15 letters, compared to baseline by week 52, according to ETDRS visual acuity chart.

130. (New) The method of claim 123, wherein five secondary doses are administered following the single initial dose.

REMARKS UNDER 37 CFR § 1.115

Formal Matters

Claims 102-130 are pending after entry of the amendments set forth herein.

Original claims 1-20 were previously canceled without prejudice. Claims 21-72 are canceled herein without prejudice.

New claims 102-130 have been added.

Support for new claims 203-240 can be found in originally pending now canceled claims 1-20, and throughout the specification.

No new matter has been added.

STATEMENT UNDER 37 C.F.R. §§1.56 AND 1.2

Applicants hereby advise the Examiner of the status of a co-pending application in compliance with the Applicant's duty to disclose under 37 C.F.R. §§1.56 and 1.2 (see also MPEP §2001.06(b)) as discussed in *McKesson Info. Soln. Inc., v. Bridge Medical Inc.*, 487 F.3d 897; 82 USPQ2d 1865 (Fed. Cir. 2007).

The Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 13/940,370, filed July 12, 2013 which issued on February 9, 2016 as U.S. Patent 9,254,338.

The Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 14/972,560, filed December 17, 2015 which issued on June 6, 2017 as U.S. Patent No. 9,669,069.

The Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 15/471,506, filed March 28, 2017 which issued on November 20, 2018 as U.S. Patent No. 10,130,681.

The Applicant wishes to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/055,847, filed August 6, 2018, which issued on December 8, 2020 as U.S. Patent No. 10,857,205.

The Applicant wishes to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/159,282, filed October 12, 2018, which issued on November 10, 2020 as U.S. Patent No. 10,828,345.

The Applicant wishes to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/397,267, filed April 29, 2019, which issued on January 12, 2021 as U.S. Patent No. 10,888,601.

The Applicant wishes to bring to the Examiner's attention co-pending U.S. Patent Application No. 17/112,404 filed December 4, 2020 for which no actions have been mailed.

The Applicant wishes to bring to the Examiner's attention co-pending U.S. Patent Application No. 17/112,063 filed December 4, 2020 for which no actions have been mailed.

The Applicant wishes to bring to the Examiner's attention co-pending U.S. Patent Application No. 17/350,958 filed June 17, 2021 for which no actions have been mailed.

The Applicant wishes to bring to the Examiner's attention co-pending U.S. Patent Application No. 17/352,892, filed June 21, 2021, which issued on February 22, 2022 as U.S. Patent No. 11,253,572.

The Applicant wishes to bring to the Examiner's attention co-pending U.S. Patent Application 17/740,744, filed May 10, 2022 for which no actions have been mailed.

The Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 13/940,370, filed July 12, 2013 which issued on February 9, 2016 as U.S. Patent 9,254,338, for which *Inter Partes* Review No. IPR2021-00881 was filed on May 5, 2021, in which a trial was instituted on November 10, 2021.

The Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 14/972,560, filed December 17, 2015 which issued on June 6, 2017 as U.S. Patent No. 9,669,069, for which *Inter Partes* Review No. IPR2021-00880 was filed on May 5, 2021, in which a trial was instituted on November 10, 2021.

The Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 13/940,370, filed July 12, 2013 which issued on February 9, 2016 as U.S. Patent 9,254,338, for which *Inter Partes* Review No. IPR2022-00298 was filed on December 9, 2021, in which a trial was instituted on February 9, 2022.

The Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 14/972,560, filed December 17, 2015 which issued on June 6, 2017 as U.S. Patent No. 9,669,069, for which *Inter Partes* Review No. IPR2022-00301 was filed on December 9, 2021, in which a trial was instituted on February 9, 2022.

The Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 13/940,370, filed July 12, 2013 which issued on February 9, 2016 as U.S. Patent 9,254,338, for which *Inter Partes* Review No. IPR2022-00258 was filed on December 9, 2021, in which a trial was instituted on February 9, 2022.

The Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 14/972,560, filed December 17, 2015 which issued on June 6, 2017 as U.S. Patent No. 9,669,069, for which *Inter Partes* Review No. IPR2022-00257 was filed on December 9, 2021, in which a trial was instituted on February 9, 2022.

The Applicant wishes to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/159,282, filed October 12, 2018, which issued on November 10, 2020 as U.S. Patent No. 10,828,345, for which Post Grant Review No. PGR2021-00035 was filed on January 7, 2021, which is terminated.

These documents are available on PAIR, and thus are not provided with this communication. Please inform the undersigned if there is any difficulty in obtaining the documents from PAIR.

CONCLUSION

Applicant submits 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, please 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-008CIPCON6.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 27 May 2022

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic, Reg. No. 28,807

Bozicevic, Field & Francis LLP
201 Redwood Shores Parkway, Suite 200
Redwood City, California 94065
Telephone: (650) 327-3400
Direct: (650) 833-7735
Facsimile: (650) 327-3231

Electronic Patent Application Fee Transmittal

Application Number:	17072417
Filing Date:	16-Oct-2020
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-008CIPCON6

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:				
INDEPENDENT CLAIMS IN EXCESS OF 3	1201	1	480	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:	45825993
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	27-MAY-2022
Filing Date:	16-OCT-2020
Time Stamp:	17:40:53
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$480
RAM confirmation Number	E20225QH41221524
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		REGN-008CIPCON6_2022-05-27_pre_amend.pdf	61858 e63954a992eb7d4f63473a139ffae7a8b80902b8	yes	9

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Preliminary Amendment	1	1
	Claims	2	6
	Applicant Arguments/Remarks Made in an Amendment	7	9

Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	38405 21efbb560af589f15b192bfddcb5127d6dd64de5	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	100263	

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.
17/072,417	10/16/2020	George D. YANCOPOULOS	REGN-008CIPCON6	7325

96387 7590 10/17/2022

Regeneron - Bozicevic, Field & Francis
201 REDWOOD SHORES PARKWAY
SUITE 200
REDWOOD CITY, CA 94065

EXAMINER
LOCKARD, JON MCCLELLAND

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
10/17/2022	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. 17/072,417	Applicant(s) YANCOPOULOS, George D.	
	Examiner JON M LOCKARD	Art Unit 1647	AIA (FITF) 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 27 May 2022.

☐ 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) 102-130 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) 102-130 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 16 October 2020 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: _____.

Notice of Pre-AIA or AIA Status

1. The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Status of Application, Amendments, and/or Claims

2. The Preliminary Amendment filed on 27 May 2022 has been entered in full. Claims 1-101 have been cancelled, and claims 102-130 have been added. Therefore, claims 102-130 are pending and the subject of this Office Action.

Information Disclosure Statement

3. The information disclosure statements (IDS) filed 16 October 2020, 17 June 2021, 08 July 2021, 03 September 2021, 24 November 2021 and 16 December 2021 have been considered by the examiner.

Double Patenting

4. 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).

5. 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).

6. 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.

7. Claims 21-49 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-26 of U.S. Patent No. 9,254,338. Although the conflicting claims are not identical, as they recite different dosing schedules, they are not patentably distinct from each other because claims 1-26 of the '338 patent are drawn to a method for treating an angiogenic 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:2, which comprises an immunoglobin-like (Ig) domain 2 of a first VEGF receptor (VEGFR1) and Ig domain 3 of a

second VEGF receptor (VEGFR2) and a multimerizing component, which is what aflibercept comprises. While the '338 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.

8. Claims 21-49 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-12 of U.S. Patent No. 9,669,069. Although the conflicting claims are not identical, as they recite different dosing schedules, they are not patentably distinct from each other because claims 1-12 of the '069 patent are drawn to a method for treating an angiogenic eye disorder, including age-related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization, comprising administering a fusion polypeptide having the amino acid sequence set forth in SEQ ID NO:2, which comprises an immunoglobin-like (Ig) domain 2 of a first VEGF receptor (VEGFR1) and Ig domain 3 of a second VEGF receptor (VEGFR2) and a multimerizing component, which is what aflibercept comprises. While the '069 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.

9. Claims 21-49 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-12 of U.S. Patent No. 10,130,681. Although the conflicting claims are not identical, as they recite different dosing schedules, they are not patentably distinct from each other because claims 1-12 of the '681 patent are drawn to a method for treating an angiogenic eye disorder, including age-related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization, comprising administering a fusion polypeptide having the amino acid sequence set forth in SEQ ID NO:2, which comprises an immunoglobin-like (Ig) domain 2 of a first VEGF receptor (VEGFR1) and Ig domain 3 of a second VEGF receptor (VEGFR2) and a multimerizing component, which is what aflibercept comprises. While the '681 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.

10. Claims 21-49 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-11 of U.S. Patent No. 10,828,345. Although the conflicting claims are not identical, as they recite different dosing schedules, they are not patentably distinct from each other because claims 1-11 of the '345 patent are drawn to a method for treating an angiogenic eye disorder, including age-related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal

neovascularization, comprising administering a VEGF antagonist, wherein the VEGF comprises an immunoglobin-like (Ig) domain 2 of Flt1 and Ig domain 3 of Flk1 and a multimerizing component, or aflibercept. While the '345 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.

11. Claims 21-49 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-47 of U.S. Patent No. 10,888,601. Although the conflicting claims are not identical, as they recite different dosing schedules, they are not patentably distinct from each other because claims 1-47 of the '601 patent are drawn to a method for treating an angiogenic eye disorder, including age-related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization, comprising administering aflibercept. While the '601 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.

12. Claims 21-49 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-30 of U.S. Patent No. 11,203,572. Although the conflicting claims are not identical, as they recite different dosing schedules, they are not patentably distinct from each other because claims 1-30 of the '572 patent are drawn to a method for treating an angiogenic eye disorder, including age-related macular degeneration and macular edema, comprising administering 2 mg of aflibercept, including secondary doses administered every 4 weeks, and tertiary doses administered every 8 weeks. While the '601 patent does not disclose the exact 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.

13. A rejection based on double patenting of the "same invention" type finds its support in the language of 35 U.S.C. 101 which states that "whoever invents or discovers any new and useful process... may obtain a patent therefor..." (Emphasis added). Thus, the term "same invention," in this context, means an invention drawn to identical subject matter. See *Miller v. Eagle Mfg. Co.*, 151 U.S. 186 (1894); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Ockert*, 245 F.2d 467, 114 USPQ 330 (CCPA 1957).

14. A statutory type (35 U.S.C. 101) double patenting rejection can be overcome by canceling or amending the claims that are directed to the same invention so they are no longer coextensive in scope. The filing of a terminal disclaimer cannot overcome a double patenting rejection based upon 35 U.S.C. 101.

15. Claims 102-130 are provisionally rejected under 35 U.S.C. 101 as claiming the same invention as that of claim 21-49 of copending Application No. 17/740,744 (reference application). This is a provisional statutory double patenting rejection since the claims directed to the same invention have not in fact been patented.

Summary

16. 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
September 29, 2022

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	17/072,417	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		09/29/2022	JML

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	17/072,417	YANCOPOULOS, George D.
	Examiner	Art Unit
	JON M LOCKARD	1647

Search Notes		
Search Notes	**Date**	**Examiner**
EAST (USPAT, US-PGPUB, EPO, DERWENT): See attached search history.	09/29/2022	JML
STN (MEDLINE, SCISEARCH, EMBASE, BIOSIS): See attached search history.	09/29/2022	JML
PALM: Inventor search.	09/29/2022	JML
IPR2021-00880 Reviewed Inter Partes Review of U.S. Pat. No. 9,669,069.	09/29/2022	JML
IPR2021-00881 Reviewed Inter Partes Review of U.S. Pat. No. 9,254,338.	09/29/2022	JML
IPR2021-00257 Reviewed Inter Partes Review of U.S. Pat. No. 9,669,069.	09/29/2022	JML
IPR2021-00258 Reviewed Inter Partes Review of U.S. Pat. No. 9,254,338.	09/29/2022	JML
IPR2021-00298 Reviewed Inter Partes Review of U.S. Pat. No. 9,254,338.	09/29/2022	JML
IPR2021-00301 Reviewed Inter Partes Review of U.S. Pat. No. 9,669,069.	09/29/2022	JML
IPR2021-01225 Reviewed Inter Partes Review of U.S. Pat. No. 10,130,681.	09/29/2022	JML
IPR2021-01226 Reviewed Inter Partes Review of U.S. Pat. No. 10,888,601.	09/29/2022	JML
IPR2021-01226 Reviewed Inter Partes Review of U.S. Pat. No. 10,828,345.	09/29/2022	JML

Interference Search			
US Class/CPC Symbol	**US Subclass/CPC Group**	**Date**	**Examiner**

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

=> d his

(FILE 'HOME' ENTERED AT 13:56:27 ON 29 SEP 2022)

FILE 'MEDLINE, SCISEARCH, EMBASE, BIOSIS' ENTERED AT 13:56:47 ON 29 SEP 2022

L1 18583 S AFLIBERCEPT OR ZALTRAP OR EYLEA OR (VEGF (W) TRAP)
L2 7255 S L1 (S) (EYE OR OCULAR OR RETINA? OR MACULAR)
L3 100 S L2 AND PD<=2011
L4 53 DUP REM L3 (47 DUPLICATES REMOVED)
L5 29 S L1 (P) ((EYE OR OCULAR) (3A) DISORDER?)
L6 8442 S L1 (P) ((MACULAR (W) EDEMA) OR (MACULAR (W) DEGENERATION) OR
L7 123 S L6 AND PD<=2011
L8 71 DUP REM L7 (52 DUPLICATES REMOVED)
E YANCOPOULOS G/AU
L9 1472 S E3 OR E4OR E8 OR E9
L10 117 S L1 AND L9
L11 46 DUP REM L10 (71 DUPLICATES REMOVED)

EAST Search History

EAST Search History (Prior Art)

/J.L./

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	9,928	(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	ON	2022/09/29 12:17
L2	1,206	l1 same ((chimer$ or fusion) same vegf)	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2022/09/29 12:17
L3	9,681	(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	ON	2022/09/29 12:17
L4	617	l3 with ((chimer$ or fusion) with vegf)	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2022/09/29 12:17
L5	12,652	aflibercept zaltrap eylea (vegf adj trap)	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2022/09/29 12:17
L6	407	(l2 l4 l5) same ((eye or ocular or retina$ or macular) with disorder)	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2022/09/29 12:18
L7	38	l6 and @py<="2013"	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2022/09/29 12:18
L8	543	yancopoulos-g$.in.	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2022/09/29 12:19
L9	93	(l2 l4 l5) and l8	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2022/09/29 12:19
L10	41	l9 and (treat treating treatment).clm.	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2022/09/29 12:19
L11	26	l6 and @py<="2011"	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2022/09/29 12:21

9/29/2022 12:33:40 PM
C:\Users\jlockard\Documents\EAST\Workspaces\17072417B.wsp

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

BIB DATA SHEET

CONFIRMATION NO. 7325

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
17/072,417	10/16/2020 RULE	424	1647	REGN-008CIPCON6

APPLICANTS

REGENERON PHARMACEUTICALS, INC., Tarrytown, NY

INVENTORS

George D. YANCOPOULOS, Yorktown Heights, NY;

**** CONTINUING DATA ***************************

This application is a CON of 16/055,847 08/06/2018 PAT 10857205
and is a CON of 16/397,267 04/29/2019 PAT 10888601
which is a CON of 16/159,282 10/12/2018 PAT 10828345
which is a CON of 15/471,506 03/28/2017 PAT 10130681
which is a CON of 14/972,560 12/17/2015 PAT 9669069
which is a CON of 13/940,370 07/12/2013 PAT 9254338
which 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 ****

10/22/2020

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	52	3

ADDRESS

Regeneron - Bozicevic, Field & Francis
201 REDWOOD SHORES PARKWAY
SUITE 200
REDWOOD CITY, CA 94065
UNITED STATES

TITLE

USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

FILING FEE RECEIVED		
5500	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

Receipt date: 06/17/2021

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	17/072,417
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	~~To Be Assigned~~ 1647
				Examiner Name	~~To Be Assigned~~ Jon Lockard
Sheet	1	of	1	Attorney Docket Number	REGN-008CIPCON6

U.S. PATENT DOCUMENTS

Examiner Initial*	Cite No.	Patent Number Number-Kind Code (*if known*)	Issue Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1				

U.S. PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No.	Publication Number **Number-Kind Code** (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1				

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No.	Foreign Document Number Country Code-Number-Kind Code (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	1					

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
/J.L/	1	HEIER, J., "Intravitreal VEGF Trap for AMD: An Update, The CLEAR-IT 2 Extension Study" Presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Retina Today (2009) pp. 44-45		

Examiner Signature	/JON M LOCKARD/	Date Considered	09/29/2022

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	17/072,417
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned 1647
				Examiner Name	To Be Assigned Jon Lockard
Sheet	1	of	1	Attorney Docket Number	REGN-008CIPCON6

U.S. PATENT DOCUMENTS

Examiner Initial*	Cite No.	Patent Number Number-Kind Code (*if known*)	Issue Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
/J.L/	1	6897294	2005-05-24	Davis-Smyth et al.	

U.S. PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No.	Publication Number **Number-Kind Code** (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1				

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No.	Foreign Document Number Country Code-Number-Kind Code (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	1					

NON PATENT LITERATURE DOCUMENTS

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

Examiner Signature	/JON M LOCKARD/	Date Considered	09/29/2022

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

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	~~To be assigned~~ 1647

U.S. PATENT DOCUMENTS					
		DOCUMENT NUMBER	DATE	NAME	REFERENCE PROVIDED*
	1.	US 2004/0213787 A1	2004-10-28	Sleeman *et al.*	not required per 69 Fed. Reg. 56481
	2.	US 6,833,349 B2	2004-12-21	Xia *et al.*	not required per 69 Fed. Reg. 56481
	3.	US 2004/0266688 A1	2004-12-30	Nayak	not required per 69 Fed. Reg. 56481
	4.	US 2005/0032699 A1	2005-02-10	Holash *et al.*	not required per 69 Fed. Reg. 56481
	5.	US 6,879,294 B2	2005-05-24	Davis-Smyth *et al.*	not required per 69 Fed. Reg. 56481
	6.	US 2005/0281822 A1	2005-12-22	Cedarbaum *et al.*	not required per 69 Fed. Reg. 56481
	7.	US 2006/0030000 A1	2006-02-09	Alitalo *et al.*	not required per 69 Fed. Reg. 56481
	8.	US 7,378,095 B2	2008-05-27	Cao *et al.*	not required per 69 Fed. Reg. 56481
	9.	US 7,482,002 B2	2009-01-27	Cedarbaum	not required per 69 Fed. Reg. 56481
	10.	US 2009/0264358 A1	2009-10-22	Yu	not required per 69 Fed. Reg. 56481
	11.	US 7,750,138 B2	2010-07-06	Fang *et al.*	not required per 69 Fed. Reg. 56481
	12.	US 7,951,585 B2	2011-05-31	Ke	not required per 69 Fed. Reg. 56481
	13.	US 8,216,575 B2	2012-07-10	Yu	not required per 69 Fed. Reg. 56481
	14.	US 2013/0295094 A1	2013-11-07	Yancopoulos	not required per 69 Fed. Reg. 56481
	15.	US 9,657,084 B2	2017-05-23	Ke *et al.*	not required per 69 Fed. Reg. 56481

FOREIGN PATENT DOCUMENTS						
		DOCUMENT NUMBER	DATE	COUNTRY	TRANSLATION	REFERENCE PROVIDED*
	16.	CN 1304427C	2007-03-14	China	Machine translation	Herewith
	17.	CN 100502945C	2009-06-24	China	Corresponds to US 2009/0264358 A1	Herewith
	18.	CN 100567325C	2009-12-09	China	Machine translation	Herewith
	19.	WO 2012/097019	2012-07-19	WIPO	N/A	Herewith
	20.	CN 102233132 B	2013-10-23	China	Machine translation	Herewith
	21.	CN 102380096 B	2014-04-30	China	Machine translation	Herewith
	22.	CN 103212075 B	2017-06-27	China	Machine translation	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

FOREIGN PATENT DOCUMENTS						
		DOCUMENT NUMBER	DATE	COUNTRY	TRANSLATION	REFERENCE PROVIDED*
	23.	CN 107115294 A	2017-09-01	China	Machine translation	Herewith

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	24.	Anonymous, Meeting Archive Titled "PA003 Eighteen-Month Results From an Extension Study of a Phase 2, Dose- and Interval-Ranging Study of VEGF Trap-Eye in Wet AMD," presented by David S Boyer, MD at Moscone Center (October 2009)	Herewith
	25.	Anonymous, Meeting Archive Titled "PA040 One-Year Results of the DA VINCI Study of VEGF Trap-Eye in Diabetic Macular Edema," presented by Diana V Do, MD at Orange County Convention Center (October 2011)	Herewith
	26.	Anonymous, Meeting Archive Titled "PA080 One-Year Results of a Phase 2 Study of Intravitreal VEGF Trap-Eye in Patients with Neovascular Age-Related Macular Degeneration," presented by David S Boyer, MD at Georgia World Congress Center (November 2008)	Herewith
	27.	Anonymous, Meeting Archive Titled "PO259 OCT and Fluorescein Angiography Outcomes Through 1 Year for a Phase 2 Study of Intravitreal VEGF Trap-Eye in Neovascular AMD," presented by Peter K Kaiser, MD at Moscone Center (October 2009)	Herewith
	28.	Anonymous, Meeting Archive Titled "PO260 VEGF Trap-Eye Vision-Specific Quality of Life Through 52 Weeks in Patients with Neovascular AMD in CLEAR-IT 2: A Phase 2 Clinical Trial," presented by Allen C Ho, MD at Moscone Center (October 2009)	Herewith
	29.	Anonymous, Meeting Archive Titled "PO492 One-Year Results of the VIEW 1 and VIEW 2 Studies: VEGF Trap-Eye in Wet AMD," presented by David M Brown MD at Orange County Center (October 2011)	Herewith
	30.	Anonymous, Meeting Archive Titled "PO549 The 6-Month (Primary Endpoint) Results of the Phase 3 GALILEO Study: VEGF Trap-Eye in Central Retinal Vein Occlusion," presented by Jean-Francois Korobelnik, MD at Orange County Convention Center (October 2011)	Herewith
	31.	Anonymous, Meeting Archive Titled "PO571 OCT and Fluorescein Angiographic Outcomes Through 1 Year for the Phase 2 Study of Intravitreal VEGF Trap-Eye in Neovascular AMD," presented by Quan Dong Nguyen, MD at Georgia World Congress Center (November 2008)	Herewith
	32.	Bontempo, "Preformulation Development of Parenteral Biopharmaceuticals," *Drugs and the Pharmaceutical Sciences*, 85:91-108 (1997)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	33.	Bressler, N. M. Treatment of Age-Related Macular Degeneration with Photodynamic Therapy Study Group, "Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: two-year results of 2 randomized clinical trials-tap report 2," *Arch. Ophthalmol.,* 119(2):198-207 (2001)	Herewith
	34.	Brown *et al.*, "Ranibizumab for Diabetic Macular Edema (DME): 24-Month Efficacy and Safety Results of RISE - a Phase 3 Randomized Controlled Trial," ARVO Annual Meeting Abstract, *Investigative Ophthalmology & Visual Science*, 52:6647 *(April 2011)*	Herewith
	35.	Brown *et al.*, "Sustained benefits from ranibizumab for macular edema following branch retinal vein occlusion: 12-month outcomes of a phase III study," *Ophthalmology,* 118(8):1594-2049 (2011)	Herewith
	36.	Cao *et al.*, "VEGF Trap Promotes Regression of Choroidal Neovascularization (CNV) and Inhibits Fibrosis and Inflammation in the Subretinal Matrigel CNV Model," ARVO Annual Meeting Abstract, *Investigative Ophthalmology & Visual Science,* 50:2979 (April 2009)	Herewith
	37.	Center for Drug Evaluation and Research Application Number: 21-756 Medical Review(s) (December 17, 2004) <URL:https://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-756_Macugen_medr.pdf>	Herewith
	38.	Center for Drug Evaluation and Research BLA Application Number: 125156 Medical Review, (June 2006) <URL:https://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/125156s0000_Lucentis_MedR.pdf>	Herewith
	39.	Cheung *et al.*, "Combined anti-PIGF and anti-VEGF Therapy Ameliorates Pathological Neovascularization and Improves Retinal Revascularization in the Murine Model of Oxygen Induced Ischemic Retinopathy," ARVO Annual Meeting Abstract, *Investigative Ophthalmology & Visual Science*, 52:6064 (April 2011)	Herewith
	40.	Dixon *et al.*, "VEGF Trap-Eye for the treatment of neovascular age-related macular degeneration," *Expert Opin. Investig. Drugs*, 18(10):1573-1580 (2009)	Herewith
	41.	The Eyetech Study Group, "Anti-Vascular Endothelial Growth Factor Therapy for Subfoveal Choroidal Neovascularization Secondary to Age-related Macular Degeneration," *Ophthalmology*, 110(5):979-986 (May 2003)	Herewith
	42.	Heier *et al.*, "Ranibizumab for Choroidal Neovascularization Secondary to Causes Other Than Age-Related Macular Degeneration: A Phase I Clinical Trial," *Ophthalmology*, 118(1):111-118 (January 2011)	Herewith
	43.	Heier, "Intravitreal Aflibercept for Diabetic Macular Edema: 148-Week Results from the VISTA and VIVID Studies," *Ophthalmology*, 123(11):2376-2385 (2016)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	44.	Herceptin label, September 1998	Herewith
	45.	Information from ClinicalTrials.gov archive on the VIEW 2 study (NCT00637377) "VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD (VIEW 2)," v1 (March 17, 2008)	Herewith
	46.	Ip *et al.*, "A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with observation to treat vision loss associated with macular edema secondary to central retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 5," *Arch. Ophthalmol.,* 127(9):1101-1114 (2009)	Herewith
	47.	Kaiser, "Vascular endothelial growth factor Trap-Eye for diabetic macular oedema," *Br. J. Ophthalmol.*, 93(2):135-36 (February 2009)	Herewith
	48.	Korobelnik *et al.*, "Intravitreal Aflibercept Injection for Macular Edema Resulting from Central Retinal Vein Occlusion," *Ophthalmology,* 121(1):202-208 (2014)	Herewith
	49.	Krzystolik *et al.*, "Prevention of Experimental Choroidal Neovascularization With Intravitreal Anti-Vascular Endothelial Growth Factor Antibody Fragment," *Arch. Ophthalmol.*, 120(3):338-346 (Mar. 2002)	Herewith
	50.	Lalwani, "All About PrONTO: Study Yielded Good Results in AMD With Treatment Guided by OCT," *Retina Today* (May 2007)	Herewith
	51.	Lobov *et al.*, "VEGF Trap Treatment Regresses Pathological Neovessels, Improves Revascularization and Reduces Retinal Ischemia in the Murine Oxygen-Induced Retinopathy (OIR) Model," ARVO Annual Meeting Abstract, *Investigative Ophthalmology & Visual Science*, 52:3128 (April 2011)	Herewith
	52.	Lucentis Approval (June 30, 2006)	Herewith
	53.	Lucentis Label Title, 7 pages, 06/2010 [Cited in Third Party Observations filed in parent application USSN 16/055,847 for which a copy is unavailable on PAIR]	Herewith
	54.	Macular Photocoagulation Study Group, "Laser photocoagulation of subfoveal neovascular lesions in age-related macular degeneration. Results of a randomized clinical trial," *Arch. Ophthalmol.,* 109(9):1220-1231 (1991)	Herewith
	55.	Mitchell *et al.*, "Ranibizumab (Lucentis) in Neovascular Age-Related Macular Degeneration: Evidence from Clinical Trials," *Brit. J. Ophthalmology,* 94:2-13 (2010) (first online publication on May 20, 2009)	Herewith
	56.	Mitra *et al.*, "Review of anti-vascular endothelial growth factor therapy in macular edema secondary to central retinal vein occlusions," *Expert Review in Ophthalmol.*, Taylor & Francis, GB 6(6):623-629 (January 2011)	Herewith
	57.	Mousa and Mousa, "Current Status of Vascular Endothelial Growth Factor Inhibition in Age-Related Macular Degeneration," *Biodrugs*, 24(3):183-194 (2010)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	58.	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*, 113(9):1522e1-1522e14 (Sept 2006) (epub July 28, 2006)	Herewith
	59.	Regeneron Pharmaceuticals Inc., "Regeneron Receives $20 Million Milestone Payment for Initiation of Phase 3 Study of VEGF Trap-Eye in Wet AMD," Media Release: 13 Aug 2007. Available from URL: http://www.regeneron.com	Herewith
	60.	Regeneron Pharmaceuticals Inc., "An Exploratory Study of the Safety, Tolerability and Biological Effect of a Single Intravitreal Administration of VEGF Trap in Patients with Diabetic Macular Edema," poster presented at the 2007 Association for Research in Vision and Ophthalmology meeting in Ft. Lauderdale, Florida (May 2007)	Herewith
	61.	Regeneron Pharmaceuticals Inc., "CLEAR-IT-2: Interim Results Of The Phase II, Randomized, Controlled Dose-and Interval-ranging Study Of Repeated Intravitreal VEGF Trap Administration In Patients With Neovascular Age-related Macular Degeneration (AMD)," poster presented at the 2007 Association for Research in Vision and Ophthalmology meeting in Ft. Lauderdale, Florida (May 2007)	Herewith
	62.	Regeneron Pharmaceuticals Inc., "Optical Coherence Tomography Outcomes of a Phase 1, Dose-Escalation, Safety, Tolerability, and Bioactivity Study of Intravitreal VEGF Trap in Patients with Neovascular Age-Related Macular Degeneration: The CLEAR-IT 1 Study," poster presented at the 2007 Association for Research in Vision and Ophthalmology meeting in Ft. Lauderdale, Florida (May 2007)	Herewith
	63.	Regeneron Pharmaceuticals Inc., "Regeneron Reports First Quarter 2008 Financial and Operating Results," Press release May 1, 2008.	Herewith
	64.	Regeneron Pharmaceuticals Inc., Form 10-Q, published November 7, 2007, for the period ending September 30, 2007.	Herewith
	65.	Regillo *et al.*, "Randomized, Double-Masked, Sham-Controlled Trial of Ranibizumab for Neovascular Age-related Macular Degeneration: OIER Study Year 1," *American Journal of Ophthalmology*, 145(2):239-248 (2008)	Herewith
	66.	Rosenfeld *et al.*, "Optical coherence tomography findings after an intravitreal injection of bevacizumab (avastin) for neovascular age-related macular degeneration," *Ophthalmic. Surg. Lasers Imaging*, 36(4):331-335 (2005)	Herewith
	67.	Scott *et al.*, "A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with standard care to treat vision loss associated with macular Edema secondary to branch retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 6," *Arch. Ophthalmol.*, 127(9):1115-1128 & 127(12):1653 (2009)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	68.	Simo and Hernandez, "Advances in Medical Treatment of Diabetic Retinopathy," *Diabetes Care*, 32(8):1556-1562 (August 2009)	Herewith
	69.	Slides for the 2008 Retina Society Meeting "VEGF Trap-Eye in Wet AMD CLEAR-IT 2: Summary of One-Year Key Results," September 28, 2008.	Herewith
	70.	Tolentino *et al.*, "One-year Results Of The Da Vinci Study of VEGF Trap-Eye In DME," ARVO Annual Meeting Abstract, *Investigative Ophthalmology & Visual Science*, 52:6646 (April 2011)	Herewith
	71.	van Bruggen *et al.*, "VEGF antagonism reduces edema formation and tissue damage after ischemia/reperfusion injury in the mouse brain," *The Journal of clinical investigation*, 104(11):1613-1620 (1999)	Herewith
	72.	WHO Drug Information, "International Nonproprietary Names for Pharmaceutical Substances (INN)," 20(2):115-119 (2006)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	To be assigned

NON-PATENT LITERATURE DOCUMENTS - INSTITUTION DECISIONS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	73.	IPR2021-00880 dated November 10, 2021, for US 9,669,069 B2	Herewith
	74.	IPR2021-00881 dated November 10, 2021, for US 9,254,338 B2	Herewith

EXAMINER	DATE CONSIDERED
/JON M LOCKARD/	09/29/2022

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	~~To Be Assigned~~ 1647
				Examiner Name	~~To Be Assigned~~ Jon Lockard
Sheet	1	of	18	Attorney Docket Number	REGN-008CIPCON6

U.S. PATENT DOCUMENTS

Examiner Initial*	Cite No.	Patent Number Number-Kind Code (*if known*)	Issue Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	7070959	2006-07-04	Papadopoulos	
	2	7303746	2007-12-04	Wiegand	
	3	7303748	2007-12-04	Wiegand	
	4	7306799	2007-12-11	Wiegand	
	5	7396664	2008-07-08	Daly et al.	
	6	8092803	2012-01-10	Furfine et al.	
	7	9254338	2016-02-09	Yancopoulos	
	8	9669069	2017-06-06	Yancopoulos	
	9	10130681	2018-11-20	Yancopoulos	
	10	10406226	2019-09-10	Dix et al.	
	11	10464992	2019-11-05	Furfine et al.	

U.S. PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No.	Publication Number **Number-Kind Code** (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	2003/0171320	2003-09-11	Guyer	
	2	2005/0163798	2005-07-28	Papadopoulos et al.	
	3	2005/0260203	2005-11-24	Wiegand et al.	
	4	2006/0058234	2006-03-16	Daly et al.	
	5	2006/0172944	2006-08-03	Wiegand et al.	
	6	2007/0190058	2007-08-16	Shams	
	7	2008/0220004	2008-09-11	Wiegand et al.	
	8	2019/0290725	2019-09-26	Vitti et al.	
	9	2019/0388539	2019-12-26	Dix et al.	
	10	2020/0017572	2020-01-16	Furfine et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No.	Foreign Document Number Country Code-Number-Kind Code (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	1	WO 2006/047325	2006-03-04	Genentech, Inc.		
	2	WO 2000/75319	2000-12-14	Regeneron Pharmaceuitcals, Inc.		
	3	WO 2004/106378 A2	2004-12-09	Regeneron Pharmaceuticals, Inc.		
	4	WO 2005/000895 A2	2005-01-05	Regeneron Pharmaceuticals, Inc.		
	5	WO 2007/022101 A2	2007-02-22	Regeneron Pharmaceuticals, Inc.		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	2	of	18	Attorney Docket Number	REGN-008CIPCON6

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No.	Foreign Document Number Country Code-Number-Kind Code (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	6	WO 2008/063932	2008-05-29	Genentech, Inc.		
	7	JP 2010-509369	2010-03-25	Genentech, Inc.	See WO 2008/063932 for English Equivalent	
	8	WO 2012/097019	2012-07-19	Regeneron Pharmaceuticals, Inc.		

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	1	16/055,847 – Third Party Submissions dated May 1, 2019		
	2	16/159,282 – Third Party Submissions dated May 31, 2019		
	3	ADSIS R&D Profile "Aflibercept: AVE 0005, AVE 005, AVE0005, VEGF Trap - Regeneron, VEGF Trap (R1R2), VEGF Trap-Eye." Drugs R D, 9(4):261-269 (2008)		
	4	ANONYMOUS "Lucentis (rangibizymab injection) Intravitreal Injection" pp. 103 (June 2006)		
	5	ANONYMOUS "Anti-VEGF 2019: The State of the Art" Review of Ophthalmology (published August 5, 2019)		
	6	BARBAZETTO, "Dosing Regimen And The Frequency Of Macular Hemorrhages In Neovascular Age-Related Macular Degeneration Treated With Ranibizumab." Retina, 30(9):1376-85 (2010)		
	7	Bayer Investor News, "Bayer and Regeneron Start additional Phase 3 Study for VEGF Trap-Eye in Wet Age-related Macular Degeneration." (May 8, 2008)		
	8	Bayer Investor News, "VEGF Trap-Eye: New Data Confirm Successes in the Treatment of Age-related Macular Degeneration" (September 28, 2008)		
	9	BENZ et al. "CLEAR-IT-2: Interim Results Of The Phase II, Randomized, Controlled Dose- and Interval-ranging Study Of Repeated Intravitreal VEGF Trap Administration In Patients With Neovascular Age-related Macular Degeneration (AMD)" ARVO Annual Meeting Abstract (May 2007)		
	10	BOYER, "A Phase IIIb Study to Evaluate the Safety of Ranibizumab in Subjects with Neovascular Age-related Macular Degeneration." Ophthalmology, 116(9):1731-39 (September 2009)		
	11	BROWN, "Ranibizumab versus Verteporfin for Neovascular Age-Related Macular Degeneration." N Engl J Med, 355(14):1432-44 (October 5, 2006)		
	12	BROWN, "Primary Endpoint Results of a Phase II Study of Vascular Endothelial Growth Factor Trap-Eye in Wet Age-related Macular Degeneration." Ophthalmology, 118(6):1089-97 (June 2011)		
	13	BROWN, "Long-term Outcomes of Ranibizumab Therapy for Diabetic Macular Edema: The 36-Month Results from Two phase III Trials." Ophthalmology, 120(10):2013-22 (October 2013)		
	14	BROWNING et al. "Aflibercept for age-related macular degeneration: a game-changer or quiet addition?" American Journal of Ophthalmology, 154(2):222-226 (August 2012)		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	3	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	15	CAMPOCHIARO et al. "Ranibizumab for Macular Edema Due to Retinal Vein Occlusions Implication of VEGF as a Critical Stimulator" Molecular Therapy, 16(4):791-799 (2008)		
	16	CAMPOCHIARO, "Ranibizumab for Macular Edema following Branch Retinal Vein Occlusion: six-month primary end point results of a phase III study." Ophthalmology, 117(6):1102-1112 (June 2010)		
	17	CAMPOCHIARO, "Sustained Benefits from Ranibizumab for Macular Edema following Central Retinal Vein Occlusion: Twelve-Month Outcomes of a phase III Study." Ophthalmology, 188(10):2041-49 (October 2011)		
	18	CAO, "A Subretinal Matrigel Rat Choroidal Neovascularization (CNV) Model and Inhibition of CNV and Associated Inflammation and Fibrosis by VEGF Trap" Investigative Ophthalmology & Visual Science, 51(11):6009- 6017 (November 2010)		
	19	CENTER FOR DRUG EVALUATION AND RESEARCH APPLICATION NUMBER: 21-756 MEDICAL REVIEW(S) (December 17, 2004) <URL:https://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-756_Macugen_medr.pdf>		
	20	CENTER FOR DRUG EVALUATION AND RESEARCH BLA APPLICATION NUMBER: 125156 MEDICAL REVIEW, (June 2006) <URL:https://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/125156s000_Lucentis_MedR.pdf>		
	21	CHARLES, Steve (Guest Lecturer) "VEGF Trap Has Positive DME Data" Tenth Annual Retina Fellows Forum Jan 29 and 30, Chicago, (Article Date 03/01/2010)		
	22	CHATZIRALLI et al. "Intravitreal aflibercept for neovascular age-related macular degeneration in patients aged 90 years or older: 2-year visual acuity outcomes" Eye (2018) 32:1523-1529		
	23	CHUNG et al. "Ziv-aflibercept: A novel angiogenesis inhibitor for the treatment of metastatic colorectal cancer" Am J Heath-Syst Pharm (November 1, 2013) 70:1887-1896		
	24	COOPER et al., "Increased Renal Expression of Vascular Endothelial Growth Factor (VEGF) and Its Receptor VEGFR-2 in Experimental Diabetes" Diabetes (1999) 48:2229-2239		
	25	CROLL et al., "VEGF-mediated inflammation precedes angiogenesis in adult brain" Experimental Neurology (2004) 187:388-402		
	26	CSAKY, "Safety Implications of Vascular Endothelial Growth Factor Blockade for Subjects Receiving Intravitreal Anti-Vascular Endothelial Growth Factor Therapies." Am. J. Ophthalmology, 148(5):647-56, (November 2009)		
	27	DeVRIESE et al., "Antibodies against Vascular Endothelial Growth Factor Improve Early Renal Dysfunction in Experimental Diabetes" J. Am. Soc. Nephrol (2001) 12:993-1000		
	28	DIXON et al., "VEGF Trap-Eye for the treatment of neovascular age-related macular degeneration" Expert Opin. Investig. Drugs, 18(10):1573-1580 (2009)		
	29	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 Ophthalmol. 93(2):144-1449 (February 2009)		
	30	DO et al., "The DA VINCI Study: phase 2 primary results of VEGF Trap-Eye in patients with diabetic macular edema" Ophthalmology, 118(9):1819-1826 (September 2011)		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	4	of	18	Attorney Docket Number	REGN-008CIPCON6

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	31	DO, "One-Year Outcomes of the DA VINCI Study of VEGF Trap-Eye in Eyes with Diabetic Macular Edema." Ophthalmology, 119(8):1658-65 (2012)		
	32	DO et al. "Results of a Phase 1 Study of Intravitreal VEGF Trap in Subjects with Diabetic Macular Edema: The CLEAR-IT DME Study" ARVO Annual Meeting Abstract (May 2007)		
	33	DO et al. "VEGF Trap-Eye Vision-specific Quality of Life through 52 Weeks in Patients with Neovascular AMD in CLEAR-IT 2: A Phase 2 Clinical Trial" ARVO Annual Meeting Abstract (April 2009)		
	34	EICHTEN, "Rapid decrease in tumor perfusion following VEGF blockade predicts long-term tumor growth inhibition in preclinical tumor models" Angiogenesis, 16:429-441 (2013)		
	35	ENGELBERT, "Treat And Extend' Dosing Of Intravitreal Antivascular Endothelial Growth Factor Therapy For Type 3 Neovascularization/Retinal Angiomatous Proliferation." Retina, 29(10):1424-31 (2009)		
	36	ENGELBERT, "Long-Term Follow-Up For Type 1 (Subretinal Pigment Epithelium) Neovascularization Using A Modified 'Treat And Extend' Dosing Regiment Of Intravitreal Antivascular Endothelial Growth Factor Therapy." Retina, 30(9):1368-75 (2010)		
	37	ENGELBERT, "The 'Treat and Extend' Dosing Regimen of Intravitreal Anti-Vascular Endothelial Growth Factor Therapy for Neovascular Age-Related Macular Degeneration." Ophthalmology Management, Issue 42, (June 2010) available at http://www.visioncareprofessional.com/emails/amdupdate/index.asp?issue=42		
	38	EREMINA et al., "Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases" Journal of Clinical Investigation (March 2003) 111(5):707-716		
	39	ERIKSSON et al., "Structure, Expression and Receptor-Binding Properties of Novel Vascular Endothelial Growth Factors" Vascular Growth Factors and Angiogenesis, Springer (1999) pp. 41-57		
	40	THE EYETECH STUDY GROUP, "Anti-Vascular Endothelial Growth Factor Therapy for Subfoveal Choroidal Neovascularization Secondary to Age-related Macular Degeneration" American Academy of Ophthalmology, 110(5):979-986 (May 2003)		
	41	Eylea®, Highlights of Prescribing Information, Revised 08/2018.		
	42	FERRARA, N. "Vascular Endothelial Growth Factor: Molecular and Biological Aspects" Advances in Organ Biology (1999) pp. 1-30		
	43	FERRARA et al., "Clinical applications of angiogenic growth factors and their inhibitors" Nature Medicine (December 1999) 5(12):1359-1364		
	44	FLYVBJERG et al., "Amelioration of Long-Term Renal Changes in Obese Type 2 Diabetic Mice by a Neutralizing Vascular Endothelial Growth Factor Antibody" Diabetes (October 2002) 51:3090-3094		
	45	FUNG, "An Optical Coherence Tomography-Guided, Variable Dosing Regiment with Intravitreal Ranibizumab (Lucentis) for Neovascular Age-related Macular Degeneration." Am J Ophthalmology, 143(4):566-83 (April 2007)		
	46	GALE, "Complementary and Coordinated Roles of the VEGFs and Angiopoietins during Normal and Pathologic Vascular Formation." Cold Spring Harbor Symposia on Quantitative Biology, Volume LXVIL, pp. 267-73 (2002)		
	47	GARCIA-QUINTANILLA, "Pharmacokinetics of Intravitreal Anti-VEGF Drugs in Age-Related Macular Degeneration." Pharmaceutics, 11:365 (2019)		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	5	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	48	GOMEZ-MANZANO, "VEGF Trap induces antiglioma effect at different stages of disease." Neuro-Oncology, 10:940-945 (December 2008)		
	49	GRAGOUDAS, "Pegaptanib for Neovascular Age-Related Macular Degeneration." N Engl J Med, 351(27):2805-16, (December 30, 2004)		
	50	GUTIERREZ et al., "Intravitreal bevacizumab (Avastin) in the treatment of macular edema secondary to retinal vein occlusion" Clin. Ophthalmol., 2(4):787,791 (2008)		
	51	HALLER et al., "VEGF Trap-Eye In CRVO: Primary Endpoint Results of the Phase 3 COPERNICUS Study" ARVO Annual Meeting Abstract (April 2011)		
	52	HEIER et al., "CLEAR-IT 2: Phase 2, Randomized Controlled Dose and Interval-Ranging Study of Intravitreal VEFG Trap Eye in Patients with Neovascular Age-Related Macular Degeneration: Predictive Factors for Visual Acuity" ARVO Annual Meeting Abstract (April 2009)		
	53	HEIER et al., " rhuFab V2 (anti-VEGF Antibody) for Treatment of Exudative AMD" Symposium 8:Experimental and Emerging Treatments for Choroidal Neovascularization, 10 pp (2002)		
	54	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)		
	55	HEIER et al., "Intravitreal Aflibercept (VEGF Trap-Eye) in Wet Age-related macular Degeneration," Ophthalmology, 119:2537-2548 (2012)		
	56	HEIER, "Intravitreal Aflibercept for Diabetic Macular Edema: 148-Week Results from the VISTA and VIVID Studies." Ophthalmology, 123(11):2376-2385 (November 2016)		
	57	HEIER et al., "The 1-year Results of CLEAR-IT 2, a Phase 2 Study of Vascular Endothelial Growth Factor Trap-Eye Dosed As-needed After 12-week Fixed Dosing" Ophthalmology 2011;118:1098–1106 (June 2011)		
	58	HEIER et al., "The 1-year Results of CLEAR-IT 2, a Phase 2 Study of Vascular Endothelial Growth Factor Trap-Eye Dosed As-needed After 12-week Fixed Dosing: Erratum" Ophthalmology 2011;118:1700 (September 2011)		
	59	HO, "VEGF Trap-Eye in Wet AMD - CLEAR-IT 2: One-Year OCT and FA Outcomes" CLEAR-IT 2 Study Group, pp 1-24 (09/28/2008)		
	60	Ho et al., Slides entitled CLEAR IT 2 One-Year Key Results, Retina Society (2008)		
	61	HOLASH et al., "Vessel Cooption, Regression, and Growth in Tumors Mediated by Angiopoietins and VEGF" Science (June 18, 1999) 284(5422):1994-1998		
	62	HOLASH, "VEGF-Trap: A VEGF blocker with potent antitumor effects" PNAS 99(17)11393-11398 (8/20/2002)		
	63	HOLASH, "Inhibitors of growth factor receptors, signaling pathways and angiogenesis as therapeutic molecular agents." Cancer Metastasis 25:243-252 (2006)		
	64	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00320775 "Safety and Tolerability of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration" 70 pages, Latest version submitted June 8, 2011 on ClinicalTrials.gov (NCT00320775_2006-2011)		
	65	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00320775 "Safety and Tolerability of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration" 10 pages, Latest version submitted March 16, 2015 on ClinicalTrials.gov (NCT00320775_2015)		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	6	of	18	Attorney Docket Number	REGN-008CIPCON6

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	66	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00320788 "Safety and Efficacy of Repeated Intravitreal Administration of Vascular Endothelial Growth Factor (VEGF) Trap in Patients With Wet Age-Related Macular Degeneration (AMD)" 71 pages, Latest version submitted December 1, 2011 on ClinicalTrials.gov (NCT00320788_2006-2011)		
	67	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00320788 "Safety and Efficacy of Repeated Intravitreal Administration of Vascular Endothelial Growth Factor (VEGF) Trap in Patients With Wet Age-Related Macular Degeneration (AMD)" 31 pages, Latest version submitted January 27, 2012 on ClinicalTrials.gov (NCT00320788_2012)		
	68	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00320814 "Phase 1 Study of VEGF Trap in Patients With Diabetic Macular Edema" 30 pages, Latest version submitted June 8, 2011 on ClinicalTrials.gov (NCT00320814_2006-2011)		
	69	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00509795 "Double-Masked Study of Efficacy and Safety of IVT VEGF Trap-Eye in Subjects With Wet AMD (VIEW 1)" 318 pages, Latest version submitted December 1, 2011 on ClinicalTrials.gov (NCT00509795_2007-2011)		
	70	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00509795 "Double-Masked Study of Efficacy and Safety of IVT VEGF Trap-Eye in Subjects With Wet AMD (VIEW 1)" 200 pages, Latest version submitted December 20, 2012 on ClinicalTrials.gov (NCT00509795_2012)		
	71	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00527423 "Randomized, Single-Masked, Long-Term, Safety and Tolerability Study of VEGF Trap-Eye in AMD" 64 pages, Latest version submitted November 1, 2011 on ClinicalTrials.gov (NCT00527423_2007-2011)		
	72	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00527423 "Randomized, Single-Masked, Long-Term, Safety and Tolerability Study of VEGF Trap-Eye in AMD" 42 pages, Latest version submitted June 10, 2013 on ClinicalTrials.gov (NCT00527423_2012-2013)		
	73	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00637377 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) (VIEW 2)" 667 pages, Latest version submitted December 16, 2011 on ClinicalTrials.gov (NCT00637377_2008-2011)		
	74	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00637377 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) (VIEW 2)" 289 pages, Latest version submitted November 28, 2014 on ClinicalTrials.gov (NCT00637377_2012-2014)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	7	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	75	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00789477 "DME And VEGF Trap-Eye [Intravitreal Aflibercept Injection (IAI;EYLEA®;BAY86-5321)] INvestigation of Clinical Impact (DA VINCI)" 135 pages, Latest version submitted May 2, 2011 on ClinicalTrials.gov (NCT00789477_2008-2011)		
	76	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00789477 "DME And VEGF Trap-Eye [Intravitreal Aflibercept Injection (IAI;EYLEA®;BAY86-5321)] INvestigation of Clinical Impact (DA VINCI)" 53 pages, Latest version submitted August 28, 2014 on ClinicalTrials.gov (NCT00789477_2013-2014)		
	77	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00943072 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)" 98 pages, Latest version submitted May 9, 2011 on ClinicalTrials.gov (NCT00943072_2009-2011)		
	78	Information from ClinicalTrials.gov archive History of Changes for Study: NCT00943072 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)" 64 pages, Latest version submitted April 16, 2013 on ClinicalTrials.gov (NCT00943072_2012-2013)		
	79	Information from ClinicalTrials.gov archive View of NCT00637377 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) (VIEW 2)" *ClinicalTrials.gov.* Web. (2010-11-30).		
	80	Information from ClinicalTrials.gov archive on the VIEW 2 study (NCT00637377) "VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD (VIEW 2)" version available (updated on 17 March 2008)		
	81	Information from ClinicalTrials.gov archive on the view of NCT00509795 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD)" (12-01-2009)		
	82	Information from ClinicalTrials.gov archive on the view of NCT00789477 "DME and VEGF Trap-Eye: Investigation of Clinical Impact" (11-18-2010)		
	83	Information from ClinicalTrials.gov archive on the view of NCT00509795 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD)" (01-07-2011)		
	84	Information from ClinicalTrials.gov archive on the view of NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 7 pages, first posted 11/13/2009; results first posted 11/22/2012; last update posted 11/3/14; printed 12/4/19 (https://clinicaltrials.gov/ct2/show/study/NCT01012973) (NOTE: May correspond to "Vascular Endothelial Growth Factor Trap‐ Eye Investigation of Efficacy and Safety in Central Retinal Vein Occlusion title, 8 pages, 11/12/2009, US **[Cited in Third Party Observations filed in parent application USSN 16/055,847 for which a copy is unavailable on PAIR]"** which was cited in the Third Party Observations dated 05/01/19)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	8	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	85	KAISER, "Vascular endothelial growth factor Trap-Eye for diabetic macular oedema." Br. J. Ophthalmol, 93(2):135-36 (February 2009)		
	86	KARIA, Niral, "Retinal vein occlusion: pathophysiology and treatment options" Clinical Ophthalmology, 4:809-816 (2010)		
	87	KOROBELNIK et al., "Intravitreal Aflibercept Injection for Macular Edema Resulting from Central Retinal Vein Occlusion" American Academy of Ophthalmology (2014) 121(1):202-208		
	88	KOROBELNIK, "Intravitreal Aflibercept for Diabetic Macular Edema." Ophthalmology, 121(11):2247-54 (November 2014)		
	89	KUO, "Comparative evaluation of the antitumor activity of antiangiogenic proteins delivered by gene transfer" PNAS 98(8):4605-4610 (04/10/2001)		
	90	KRZYSTOLIK et al., "Prevention of Experimental Choroidal NEovascularization With Intravitreal Anti-Vascular Endothelial Growth Factor Antibody Fragment" Arch Ophthamol., 120:338-346 (Mar. 2002)		
	91	LALWANI, "All About PrONTO: Study Yielded Good Results in AMD With Treatment Guided by OCT." Retina Today (May 2007)		
	92	LALWANI, 'A Variable-dosing Regimen with Intravitreal Ranibizumab for Neovascular Age-related Macular Degeneration: Year 2 of the PrONTO Study." Am J Ophthalmology, 148(1):43-58 (July 2009)		
	93	LEVINE, "Macular Hemorrhage In Neovascular Age-Related Macular Degeneration After Stabilization With Antiangiogenic Therapy." Retina, 29(8):1074-79 (2009)		
	94	Lucentis Label Title, 7 pages, 30/06/2010 [**Cited in Third Party Observations filed in parent application USSN 16/055,847 for which a copy is unavailable on PAIR**]		
	95	MAJOR et al., "DA VINCI: DME and VEGF Trap-Eye: Investigation of Clinical Impact: Phase 2 Study in Patients with Diabetic Macular Edema (DME)" ARVO Annual Meeting Abstract (April 2010)		
	96	MARGOLIS, "Hemorrhagic Recurrence Of Neovascular Age-Related Macular Degeneration Not Predicted By Spectral Domain Optical Coherence Tomography." Retinal Cases & Brief Reports, 4:1-4 (2010)		
	97	MASSIN, "Safety and Efficacy of Ranibizumab in Diabetic Macular Edema (RESOLVE Study*)." Diabetes Care, 33(11):2399-405 (November 2010)		
	98	MITCHELL, "The RESTORE Study, Ranibizumab Monotherapy or Combined with Laser versus Laser Monotherapy for Diabetic Macular Edema." Ophthalmology, 188(4):615-25 (April 2011)		
	99	MITCHELL, Edith P. "Targeted Therapy for Metastatic Colorectal Cancer: Role of Aflibercept" Clinical Colorectal Cancer (2013) 12(2):73-85		
	100	MITRA et al., "Review of anti-vascular endothelial growth factor therapy in macular edema secondary to central retinal vein occlusions" Expert Review in Ophthalmol, Taylor & Francis, GB 6(6):623-629 (January 2011)		
	101	MOUSA AND MOUSA, "Current Status of Vascular Endothelial Growth Factor Inhibition in Age-Related Macular Degeneration" Biodrugs; 24(3); 183-194 (2010)		
	102	N/A "Materials from June 2011 FDA Committee Mtg" (06/17/2011)		
	103	N/A "Materials from Dec 2011 FDA Committee Mtg"(12/01/2011)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	9	of	18	Attorney Docket Number	REGN-008CIPCON6

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	104	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)		
	105	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, 113(9):1522e1-1522e14 (Sept 2006) (epub July 28,2006)		
	106	NGUYEN et al., "Randomized, Double-masked, Active-controlled Phase 3 Trial of the Efficacy and Safety of Intravitreal VEGF Trap-Eye in Wet AMD: One-year Results of the VIEW 1 Study" ARVO Annual Meeting Abstract (April 2011)		
	107	NGUYEN, "Ranibizumab for Diabetic Macular Edema, Results from 2 Phase III Randomized Trials: RISE and RIDE." Ophthalmology, 119(4):789-801 (April 2012)		
	108	NGUYEN et al., "Results of a Phase I, Dose-Escalation, Safety, Tolerability, and Bioactivity Study of Intravitreous VEGF Trap in Patients with Neovascular Age-Related Macular Degeneration" ARVO Annual Meeting Abstract (May 2006)		
	109	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, 2003)		
	110	NOGUERA-TROISE et al., "Blockade of D114 inhibits tumour growth by promoting non-productive angiogenesis" Nature (December 2006) 444:1032-1037		
	111	OHR, "Aflibercept in wet age-related macular degeneration: a perspective review" Ther. Adv. Chronic Dis., 3(4):153-161 (2012)		
	112	OLIVERA et al., "VEGF Trap R1R2 suppresses experimental corneal angiogenesis" European Journal of Ophthalmology, 20(1):48-54 (January 1, 2010)		
	113	PAI et al., "Current concepts in intravitreal drug therapy for diabetic retinopathy" Saudi Journal of Ophthalmology 24(4):143-149 (June 30, 2010)		
	114	PAPADPPOULOS, "Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab" Angiogenesis, 15:171-185 (2012)		
	115	Regeneron SEC Form 10-K (February 27, 2008)		
	116	Regeneron SEC Form 10-K (February 26, 2009)		
	117	Regeneron SEC Form 10-K (February 17, 2011)		
	118	Regeneron SEC Form 10-Q (May 8, 2006)		
	119	Regeneron SEC Form 10-Q (August 8, 2006)		
	120	Regeneron SEC Form 10-Q (November 6, 2006)		
	121	Regeneron SEC Form 10-Q (May 4, 2007)		
	122	Regeneron SEC Form 10-Q (August 3, 2007)		
	123	Regeneron SEC Form 10-Q (April 30, 2009)		
	124	Regeneron SEC Form 10-Q (November 3, 2009)		
	125	Regeneron SEC Form 10-Q (April 29, 2010)		
	126	Regeneron SEC Form 10-Q (July 28, 2010)		
	127	Regeneron SEC Form 10-Q (October 28, 2010)		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	10	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	128	Regeneron SEC Form 10-Q (May 3, 2011)		
	129	Regeneron SEC Form 10-Q (July 28, 2011)		
	130	Regeneron SEC Form 10-Q (October 27, 2011)		
	131	Regeneron SEC Form 8-K Exhibit: "Press Release of Regeneron Pharmaceuticals, Inc. dated May 1, 2006" (May 2, 2006)		
	132	Regeneron SEC Form 8-K Exhibit: "Press Release of Regeneron Pharmaceuticals, Inc. dated May 3, 2006" (May 5, 2006)		
	133	Regeneron SEC Form 8-K Exhibit: "Slides presented at the Company's 2006 Annual Meeting of Shareholders held on June 9, 2006" (June 9, 2006)		
	134	Regeneron SEC Form 8-K Exhibit: "Press Release dated May 2, 2007" (May 3, 2007)		
	135	Regeneron SEC Form 8-K Exhibit: "Overheads for presentation at Regeneron's Annual Meeting of Shareholders to be held on June 8, 2007" (June 8, 2007)		
	136	Regeneron SEC Form 8-K Exhibit: "Press Release dated October 1, 2007" (October 1, 2007)		
	137	Regeneron SEC Form 8-K Exhibit: "Press Release dated November 6, 2007" (November 6, 2007)		
	138	Regeneron SEC Form 8-K Exhibit: "Press Release dated May 1, 2008" (May 2, 2008)		
	139	Regeneron SEC Form 8-K Exhibit: "Press Release dated November 4, 2008" (November 4, 2008)		
	140	Regeneron SEC Form 8-K Exhibit: "99(a) Slides that Regeneron Pharmaceuticals, Inc. intends to use in conjunction with meetings with investors at the J.P. Morgan 27th Annual Healthcare Conference in San Francisco on January 12-15, 2009." (January 9, 2009)		
	141	Regeneron SEC Form 8-K Exhibit: "Press Release dated April 30, 2009" (May 1, 2009)		
	142	Regeneron SEC Form 8-K Exhibit: "Press Release dated November 3, 2009." (November 4, 2009)		
	143	Regeneron SEC Form 8-K Exhibit: "Press Release Reporting Positive Results for VEGF Trap-Eye in Phase 3 Study in Central Retinal Vein Occlusion (CRVO) and in Phase 2 Study in Diabetic Macular Edema (DME) dated December 20, 2010." (December 20, 2010)		
	144	Regeneron SEC Form 8-K Exhibit: "Press Release dated February 17, 2011" (February 18, 2011)		
	145	Regeneron SEC Form 8-K Exhibit: "Press Release Reporting Positive Results for VEGF Trap-Eye in Second Phase 3 Study in Central Retinal Vein Occlusion, dated April 27, 2011" (April 27, 2011)		
	146	Regeneron SEC Form 8-K Exhibit: "Press Release dated May 3, 2011." (May 3, 2011)		
	147	Regeneron SEC Form 8-K Exhibit: "Press Release, dated June 17, 2011, Announcing that EYLEA™ (aflibercept ophthalmic solution) Received Unanimous Recommendation for Approval for Treatment of Wet AMD from FDA Advisory Committee." (June 21, 2011)		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	11	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	148	Regeneron SEC Form 8-K Exhibit: "Presentation entitled VEGF Trap-Eye in CRVO: 1-year Results of the Phase 3 COPERNICUS Study" (August 22, 2011)		
	149	Regeneron SEC Form 8-K Exhibit: "Press Release Announcing FDA Approval of EYLEA™ (aflibercept) Injection for the Treatment of Wet Age-Related Macular Degeneration, dated November 18, 2011" (November 21, 2011)		
	150	Regeneron Press Release "Positive Interim Phase 2 Data Reported For VEGF Trap-Eye In Age-Related Macular Degeneration" (March 27, 2007)		
	151	Regeneron Press Release "VEGF TRAP-Eye Phase 2 Wet AMD Results Reported At Arvo Annual Meeting" (May 9, 2007)		
	152	Regeneron Press Release "Regeneron Reports Second Quarter Financial And Operating Results" (August 1, 2007)		
	153	Regeneron Pharmaceuticals, Inc., "Regeneron and Bayer Healthcare Initiate Phase 3 Global Development Program for VEGF Trap-Eye In Wet Age-Related Macular Degeneration (AMD)" (August 2, 2007)		
	154	Regeneron Pharmaceuticals, Inc. FORM 10-Q, published on 7 November 2007 for the period ending 30 September 2007		
	155	Regeneron Press Release "Regeneron Announces Positive Primary Endpoint Results From A Phase 2 Study Of VEGF Trap-Eye In Age-Related Macular Degeneration" (October 1, 2007)		
	156	Regeneron Press Release "Regeneron Reports Fourth Quarter And Full Year 2007 Financial And Operating Results" (February 27, 2008)		
	157	Regeneron Pharmaceuticals, Inc., "Regeneron and Bayer HealthCare Announce Encouraging 32-Week Follow-up Results from a Phase 2 Study of VEGF Trap-Eye in Age-Related Macular Degeneration" (April 28, 2008)		
	158	Regeneron, Press release "Regeneron Reports First Quarter 2008 Financial and Operating Results", May 1, 2008.		
	159	Regeneron Press Release, "Bayer and Regeneron Dose First Patient in Second Phase 3 Study for VEGF Trap-Eye in Wet Age-Related Macular Degeneration." May 8, 2008		
	160	Regeneron Pharmaceuticals Inc., "CLEAR-IT-2: Interim Results Of The Phase II, Randomized, Controlled Dose-and Interval-ranging Study Of Repeated Intravitreal VEGF Trap Administration In Patients With Neovascular Age-related Macular Degeneration (AMD)" poster presented at the 2007 Association for Research in Vision and Ophthalmology meeting in Ft. Lauderdale, Florida (May 2007)		
	161	Regeneron Pharmaceuticals Inc., "An Exploratory Study of the Safety, Tolerability and Biological Effect of a Single Intravitreal Administration of VEGF Trap in Patients with Diabetic Macular Edema" poster presented at the 2007 Association for Research in Vision and Ophthalmology meeting in Ft. Lauderdale, Florida (May 2007)		
	162	Regeneron Pharmaceuticals Inc., "Optical Coherence Tomography Outcomes of a Phase 1, Dose-Escalation, Safety, Tolerability, and Bioactivity Study of Intravitreal VEGF Trap in Patients with Neovascular Age-Related Macular Degeneration: The CLEAR-IT 1 Study" poster presented at the 2007 Association for Research in Vision and Ophthalmology meeting in Ft. Lauderdale, Florida (May 2007)		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	12	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	163	Regeneron Pharmaceuticals, Inc., "Regeneron and Bayer HealthCare Announce VEGF Trap-Eye Achieved Durable Improvement in Vision over 52 Weeks in a Phase 2 Study in Patients with Age-related Macular Degeneration" (August 19, 2008)		
	164	Regeneron Pharmaceuticals Inc., " VIEW 1 Vascular Endothelial Growth Factor (VEGF) Trap-Eye 1-Year Results: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) " presented at Bascom Palmer Eye Institute's Angiogenesis, Exudation and Degeneration 2011 meeting in Miami, Florida (February 12, 2011)		
	165	Regeneron Pharmaceuticals Inc., " VIEW 2 Vascular Endothelial Growth Factor (VEGF) Trap-Eye 1-Year Results: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) " presented at Bascom Palmer Eye Institute's Angiogenesis, Exudation and Degeneration 2011 meeting in Miami, Florida (February 12, 2011)		
	166	Regeneron Pharmaceuticals Inc., "VEGF Trap-Eye CLEAR-IT 2 Final Primary Endpoint Results" presented at the 2007 Retina Society Conference in Boston, Massachusetts (September 30, 2007)		
	167	Regeneron Pharmaceuticals Inc., "VEGF Trap-Eye Final Phase 2 Results in Age-related Macular Degeneration Presented at 2008 Retina Society Meeting" (September 28, 2008) (XP-002770952)		
	168	Regeneron 2008 Annual Report		
	169	Regeneron Pharmaceuticals, Inc. "Regeneron Reports Full Year and Fourth Quarter 2008 Financial and Operating Results" (February 26, 2009)		
	170	Regeneron Pharmaceuticals, Inc. "Bayer and Regeneron Extend Development Program for VEGF Trap-Eye to Include Central Retinal Vein Occlusion" (April 30, 2009)		
	171	Regeneron Press Release "First Patient Enrolled In Regeneron And Bayer Healthcare VEGF Trap-Eye Phase 3 Program In Central Retinal Vein Occlusion" (July 23, 2009)		
	172	Regeneron Press Release "Enrollment Completed in Regeneron and Bayer HealthCare Phase 3 Studies of VEGF Trap-Eye in Neovascular Age-Related Macular Degeneration (Wet AMD)" September 14, 2009		
	173	Regeneron 2009 Annual Report and 10-K		
	174	Regeneron Press Release, "VEGF Trap-Eye Shows Positive Results in a Phase 2 Study in Patients With Diabetic Macular Edema." February 18, 2010		
	175	Regeneron Press Release "Regeneron Schedules November 22, 2010 Teleconference And Webcast To Discuss Results Of Two Phase 3 Studies With VEGF Trap-Eye In Wet Age-Related Macular Degeneration" (November 19, 2010)		
	176	Regeneron Press Release "Bayer and Regeneron Report Positive Top-Line Results of Two Phase 3 Studies with VEGF Trap-Eye in Wet Age-related Macular Degeneration" November 22, 2010		
	177	Regeneron Press Release "Regeneron and Bayer Report Positive Results for VEGF Trap-Eye in Phase 3 Study in Central Retinal Vein Occlusion (CRVO) and in Phase 2 Study in Diabetic Macular Edema (DME)" December 20, 2010		
	178	Regeneron 2010 Annual Report and 10-K		
	179	Regeneron Press Release "Regeneron And Bayer Start Phase 3 Trial To Extend Ophthalmology Research & Development Program For VEGF Trap-Eye In Asia" (January 18, 2011)		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	13	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	180	Regeneron Press Release "Regeneron To Webcast Investor Briefing On VEGF Trap-Eye Clinical Program On Sunday, February 13th At 9 Am Et" (February 9, 2011)		
	181	Regeneron Press Release "Regeneron Submits Biologics License Application To FDA For VEGF Trap-Eye For Treatment Of Wet Age-Related Macular Degeneration" (February 22, 2011)		
	182	Regeneron Press Release "Regeneron And Bayer Announce Start Of Phase 3 Clinical Program In Diabetic Macular Edema" (April 8, 2011)		
	183	Regeneron Pharmaceuticals, Inc., "FDA Grants Priority Review for VEGF Trap-Eye for the Treatment of Wet Age-Related Macular Degeneration" (April 18, 2011)		
	184	Regeneron Press Release "VEGF Trap-Eye Submitted for EU Marketing Authorization for Treatment of Wet Age-Related Macular Degeneration (June 7, 2011)		
	185	Regeneron Pharmaceuticals, Inc., "Regeneron Announces EYLEA™ (aflibercept ophthalmic solution) Receives Unanimous Recommendation for Approval for Treatment of Wet AMD from FDA Advisory Committee" (June 17, 2011)		
	186	Regeneron Press Release "Regeneron Announces Clinical Presentations at ASRS 2011 Annual Meeting" (August 17, 2011)		
	187	Regeneron Pharmaceuticals, Inc., "Regeneron Announces FDA Approval of EYLEA™ (aflibercept) Injection for the Treatment of Wet Age-Related Macular Degeneration: CORRECTED (November 18, 2011)		
	188	Regeneron Pharmaceuticals, Inc., "Regeneron and Bayer Initiate Phase 3 Clinical Program for the Treatment of Wet Age-Related Macular Degeneration in China" (November 28, 2011)		
	189	Regeneron Pharmaceuticals, Inc., "Two Year Results of Phase 3 Studies with EYLEA™ (aflibercept) Injection in wet AMD Show Sustained Improvement in Visual Acuity" (December 5, 2011)		
	190	REGILLO et al., "Randomized, Double-Masked, Sham-Controlled Trial of Ranibizumab for Neovascular Age-related Macular Degeneration: OIER Study Year 1" American Journal of Ophthalmology, 145(2):239-248 (2008)		
	191	ROSENFELD, "Ranibizumab for Neovascular Age-Related Macular Degeneration." N Engl J Med, 355(14):1419-31 (October 5, 2006)		
	192	ROSENFELD, "Lessons Learned From Avastin and OCT-The Great, the Good, the Bad, and the Ugly: The LXXV Edward Jackson Memorial Lecture." Am. J. Ophthalmology, 204:26-45 (August 2019)		
	193	RUDGE et al., "VEGF Trap complex formation measures production rates of VEGF, providing a biomarker for predicting efficacious angiogenic blockade" PNAS (November 20, 2007) 104(47):18363-18370		
	194	RUDGE et al. "Clinical Development of VEGF Trap" In: Figg W.D., Folkman J. (eds) Angiogenesis (2008)		
	195	SCHMIDT-ERFURTH, "Efficacy and Safety of Monthly versus Quarterly Ranibizumab Treatment in Neovascular Age-related Macular Degeneration: The EXCIE Study" Ophthalmology, 118(5)831-839 (2010)		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	14	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	196	SCHMIDT-ERFURTH et al. "Primary Results of an International Phase III Study Using Intravitreal VEGF Trap-Eye Compared to Ranibizumab in Patients with Wet AMD (VIEW 2)" ARVO Annual Meeting Abstract (April 2011)		
	197	SCHMIDT-ERFURTH, "Three-Year Outcomes of Individualized Ranibizumab Treatment in Patients with Diabetic Macular Edema." Ophthalmology, 121(5):1045-53,(May 2014)		
	198	SCHMIDT-ERFURTH et al., "Intravitreal Aflibercept Injection for Neovascular Age-related Macular Degeneration" Ophthalmology (2014) 121:193-201		
	199	SCHNICHELS, "Comparative toxicity and proliferation testing of aflibercept, bevacizumab and ranibizumab on different ocular cells." Br. J. Ophthalmol., 97:917-923 (2013)		
	200	SEMERARO et al., "Aflibercept in wet AMD: specific role and optimal use" Drug Design, Development and Therapy (August 2, 2013) 7:711-722		
	201	SHARMA and S. AND KAISER, P. K., Update on VEGF TRAP-Eye Clinical Trials and Retinal. Physician, pp. 1-6 (Nov/Dec 2010) <URL: https://www.retinalphysician.com/issues/2010/nov-dec/update-on-vegf-trap-eye-clinical-trials>		
	202	SIMO AND HERNANDEZ, "Advances in Medical Treatment of Diabetic Retinopathy" Diabetes Care, 32(8):1556-1562 (August 2009)		
	203	SLAKTER et al., "Influence of Baseline Angiographic Classification on Outcomes in the CLEAR-IT 2 Phase 2 Study of Intravitreal VEGF Trap-Eye in Neovascular Age-Related Macular Degeneration" ARVO Annual Meeting Abstract (April 2010)		
	204	SLAKTER et al., "A Phase 2, Randomized, Controlled Dose-and Interval-Ranging Study of Intravitreal VEGF Trap-Eye in Patients with Neovascular Age-Related Macular Degeneration: Optical Coherence Tomography (OCT) and Fluorescein Angiography (FA) Outcomes at 1 Year" ARVO Annual Meeting Abstract (April 2009)		
	205	Slides for the 2008 Retina Society Meeting "VEGF Trap-Eye in Wet AMD CLEAR-IT 2: Summary of One-Year Key Results", September 28, 2008.		
	206	SPAIDE, "Ranibizumab According to Need: A Treatment for Age-related Macular Degeneration." Am J Ophthalmology, 143(4):679-680 (April 2007)		
	207	STEWART, "The expanding role of vascular endothelial growth factor inhibitors in opthamology" Mayo Clin Proc. 87(1):77-88 (January 2012)		
	208	STEWART et al., "Predicted biological activity of intravitreal VEGF Trap" British Journal of Ophthalmology, 92(5):667-668 (2008)		
	209	STEWART, "Aflibercept" Nature Reviews: Drug Discovery 11:269-270 (04/01/2012)		
	210	TANNOCK et al., "Aflibercept versus placebo in combination with docetaxel and prednisone for treatment of men with metastatic castration-resistant prostate cancer (VENICE): a phase 3, double-blind randomized trial" Lancet Oncol (2013) 14:760-768		
	211	THOMAS REUTERS INTEGRITY "VEGF Trap-Eye final phase II results in age-related macular degeneration presented at 2008 Retina Society Meeting" (September 28, 2008)		
	212	THURSTON, Gavin "Complementary actions of VEGF and Angiopoietin-1 on blood vessel growth and leakage" J. Anat. (2002) 200:575-580		
	213	THURSTON, "Vascular endothelial growth factor and other signaling pathways in developmental and pathologic angiogenesis." International Journal of Hematology, 80:7-20 (2004)		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	15	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS		
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T
	214	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 38 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_01182013_27424.1)	
	215	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_01252011_27433.1)	
	216	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 11 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_01262012_27428.1)	
	217	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 38 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_01302013_27423.1)	
	218	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_02092010_27442.1)	
	219	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 11 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_02202012_27427.1)	
	220	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_03162010_27441.1)	
	221	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_04082011_27432.1)	
	222	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_04162010_27440.1)	

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	16	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	223	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_06232011_27431.1)		
	224	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_07222010_27439.1)		
	225	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_08252010_27438.1)		
	226	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_08262010_27437.1)		
	227	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_09082010_27436.1)		
	228	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_09192011_27430.1)		
	229	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_10042010_27435.1)		
	230	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 38 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_10232012_27426.1)		
	231	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 38 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_10272013_27422.1)		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	17	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	232	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_11012010_27434.1)		
	233	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_11132009_27444.1)		
	234	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 10 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_11292011_27429.1)		
	235	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 38 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_12182012_27425.1)		
	236	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_12212010_27443.1)		
	237	Vascular Endothelial Growth Factor Trap‐ Eye Investigation of Efficacy and Safety in Central Retinal Vein Occlusion title, 8 pages, 11/12/2009, US **[Cited in Third Party Observations filed in parent application USSN 16/055,847 for which a copy is unavailable on PAIR] NOTE:** May correspond to **"**Information from ClinicalTrials.gov archive on the view of NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 7 pages, first posted 11/13/2009; results first posted 11/22/2012; last update posted 11/3/14; printed 12/4/19 (https://clinicaltrials.gov/ct2/show/study/NCT01012973)" cited by the Examiner in the Office Action dated 12/10/19 in USSN 16/055,847		
	238	WACHSBERGER, "VEGF trap in combination with radiotherapy improves tumor control in u87 glioblastoma." Int. J. Radiation Oncology Biol Phys. 67(5):1526-1537 (2007)		
	239	WHO Drug Information, "International Nonproprietary Names for Pharmaceutical Substances (INN)" 20(2):115-119 (2006)		
	240	WOLFSON, "Regeneron Focuses on Age-Related Macular Degeneration." Chemistry & Biology 15:303-304 (April 2008)		
	241	XIA et al., "Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis" Blood (July 1, 2003) 102(1):161-168		
	242	YANCOPOULOS, "Vascular-specific growth factors and blood vessel formation." Nature 407:242-48 (September 14, 2000)		

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	To Be Assigned
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	18	of	18	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	243	YANCOPOULOS, "Clinical Application of Therapies Targeting VEGF." Cell 143:13-16 (October 1, 2010)		
	244	YUNG, "Moving Toward the Next Steps in Angiogenesis Therapy?" Society for Neuro-Oncology, 10:939 (2008)		

Examiner Signature	/JON M LOCKARD/	Date Considered	09/29/2022

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	17/072,417
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	1	of	2	Attorney Docket Number	REGN-008CIPCON6

U.S. PATENT DOCUMENTS

Examiner Initial*	Cite No.	Patent Number Number-Kind Code (*if known*)	Issue Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1				

U.S. PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No.	Publication Number **Number-Kind Code** (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1				

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No.	Foreign Document Number Country Code-Number-Kind Code (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	1					

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	1	Eylea®, Highlights of Prescribing Information, Revised 11/2011		
	2	IPR2021-00880, Paper 1, Petition for IPR (May 5, 2021)		
	3	IPR2021-00880, Exhibit 1002, Albini Declaration (May 4, 2021)		
	4	IPR2021-00880, Exhibit 1003, Gerritsen Declaration (April 30, 2021)		
	5	IPR2021-00880, Paper 10, Preliminary Response of Patent Owner (August 16, 2021)		
	6	IPR2021-00881, Paper 1, Petition for IPR (May 5, 2021)		
	7	IPR2021-00881, Exhibit 1002, Albini Declaration (May 4, 2021)		
	8	IPR2021-00881, Exhibit 1003, Gerritsen Declaration (April 26, 2021)		
	9	IPR2021-00881, Paper 10, Preliminary Response of Patent Owner (August 16, 2021)		
	10	IPR2021-00881, Exhibit 2001, Do Declaration (August 13, 2021)		
	11	Mitchell *et al.*, "Evaluating the Impact of Intravitreal Aflibercept on Diabetic Retinopathy Progression in the VIVID-DME and VISTA-DME Studies" Ophthalmol Retina 2(10):988-96 (2018)		
	12	PGR2021-00035, Paper 2, Petition for PGR (January 7, 2021)		
	13	PGR2021-00035, Paper 6, Preliminary Response of Patent Owner (April 15, 2021)		
	14	PGR2021-00035, Exhibit 1003 Wu Declaration (January 7, 2021)		
	15	PGR2021-00035, Exhibit 2001 Do Declaration (April 14, 2021)		
	16	PGR2021-00035, Exhibit 2002 D. Brown Declaration (April 14, 2021)		
	17	CAO, J. R., R.; Wang, Q.; Yancopoulos, G.D.; Wiegand, S.J. (2002). Inhibition of Corneal Neovascularization and Inflammation by VEGF Trap. In "ARVO", Invest. Ophthalmol. Vis. Sci. Vol. 43. E-Abstract 1863		

Examiner Signature		Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	17/072,417
				Filing Date	2020-10-16
				First Named Inventor	George D. YANCOPOULOS
				Art Unit	To Be Assigned
				Examiner Name	To Be Assigned
Sheet	2	of	2	Attorney Docket Number	REGN-008CIPCON6

	NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		T
	18	WANG, Q. R., R.; Cao, J.; Yancopoulos, G.D.; and Wiegand, S.J. (2002). Anti-Angiogenic Properties of a New VEGF Antagonist, VEGF Trap, in a Mouse Model of Retinal Neovascularization. In "ARVO", Invest. Ophthalmol. Vis. Sci., Vol. 43. E-Abstract. 3714		
	19	SAISHIN, Y., Saishin, Y., Takahashi, K., Lima e Silva, R., *et al.* (2003). VEGF-TRAP(R1R2) suppresses choroidal neovascularization and VEGF-induced breakdown of the blood-retinal barrier. J Cell Physiol 195:241-48		
	20	CURSIEFEN, C., Cao, J., Chen, L., Liu, Y., Maruyama, K., *et al.* (2004). Inhibition of hemangiogenesis and lymphangiogenesis after normal-risk corneal transplantation by neutralizing VEGF promotes graft survival. Invest Ophthalmol Vis Sci 45(8):2666-73		
	21	CURSIEFEN, C., Chen, L., Borges, L. P., Jackson, D., Cao, J., *et al.* (2004). VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. J Clin Invest 113(7):1040-50		
	22	CAO, J.; Song, H.; Renard, R.A.; Liu, Y.; Yancopolous, G.D.; Wiegand, S.J. (2005). Systemic Administration of VEGF Trap Suppresses Vascular Leak and Leukostasis in the Retinas of Diabetic Rats. In "ARVO", Vol. 46. Invest. Ophthalmol. Vis. Sci. E-Abstract 446		
	23	NORK, T. M., Dubielzig, R. R., Christian, B. J., Miller, P. E., Miller, J. M., *et al.* (2011). Prevention of experimental choroidal neovascularization and resolution of active lesions by VEGF trap in nonhuman primates. Arch Ophthalmol 129(8):1042-52		

Examiner Signature	/JON M LOCKARD/	Date Considered	09/29/2022

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

Inventor Information for 17/072417

/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 Gr

Search Another: Application # Search **or Patent #** Search **or International Registration #** Search

PCT / / Search **or PG PUBS #** Search

Attorney Docket # Search

Bar Code # Search

To Go BACK Use BACK Button on Your BROWSER Tool Bar
Back to PALM | ASSIGNMENT | OASIS | Home page

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

U.S. PATENT DOCUMENTS					
		DOCUMENT NUMBER	DATE	NAME	REFERENCE PROVIDED*
	1	6,171,586	1/9/2001	Lam *et al.*	not required per 69 Fed. Reg. 56481
	2	7,303,747	12/4/2007	Wiegand *et al.*	not required per 69 Fed. Reg. 56481
	3	7,374,757	5/20/2008	Papadopoulos *et al.*	not required per 69 Fed. Reg. 56481
	4	7,374,758	5/20/2008	Papadopoulos *et al.*	not required per 69 Fed. Reg. 56481
	5	7,378,095	5/27/2008	Cao *et al.*	not required per 69 Fed. Reg. 56481
	6	7,521,049	4/21/2009	Wiegand *et al.*	not required per 69 Fed. Reg. 56481
	7	7,531,173	5/12/2009	Wiegand *et al.*	not required per 69 Fed. Reg. 56481
	8	10,828,345	11/10/2020	Yancopoulos	not required per 69 Fed. Reg. 56481
	9	2003/0113316	6/19/2003	Kaisheva *et al.*	not required per 69 Fed. Reg. 56481
	10	2003/0138417	7/24/2003	Kaisheva *et al.*	not required per 69 Fed. Reg. 56481
	11	2004/0197324	10/7/2004	Liu *et al.*	not required per 69 Fed. Reg. 56481
	12	2006/0217311	9/28/2006	Dix *et al.*	not required per 69 Fed. Reg. 56481
	13	2016/0130337	5/12/2016	Gekkieva *et al.*	not required per 69 Fed. Reg. 56481

FOREIGN PATENT DOCUMENTS						
		DOCUMENT NUMBER	DATE	COUNTRY	TRANSLATION	REFERENCE PROVIDED*
	14	2663325	11/20/2013	EP	n/a	Herewith
	15	97/04801	2/13/1997	WO	n/a	Herewith

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	16	7,374,758 – Patent Term Extension Application submitted December 22, 2011	Herewith
	17	ADIS R&D Profile "Aflibercept: AVE 0005, AVE 005, AVE0005, VEGF Trap - Regeneron, VEGF Trap (R1R2), VEGF Trap-Eye." Drugs R D, 9(4):261-269 (2008)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	18	Andersen & Krummen, "Recombinant protein expression for therapeutic applications" Current Opinion in Biotechnology 13:117-123 (2002)	Herewith
	19	Anderson *et al.*, "Delivery of Anti-Angiogenic Molecular Therapies for Retinal Disease" Drug Discovery Today 15: 272 (2010)	Herewith
	20	Article in Retinal Physician, "Subspecialty News", available online at http://www.retinalphysician.com/printarticle.aspx?articleID=104007 (March 2010)	Herewith
	21	Ass'n for Res. Vision & Ophthalmology, ARVO® News (Summer 2007)	Herewith
	22	Ass'n for Res. Vision & Ophthalmology, ARVO® News (Winter/Spring 2008)	Herewith
	23	AVASTIN® label	Herewith
	24	Avery, R. L., D. J. Pieramici, M. D. Rabena, A. A. Castellarin, M. A. Nasir and M. J. Giust, "Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration" Ophthalmology 113(3): 363-372 e365 (2006)	Herewith
	25	Bashshur *et al.*, "Intravitreal Bevacizumab for the Management of Choroidal Neovascularization in Age-Related Macular Degeneration" Am J. Ophthalmology 142: 1 (2006)	Herewith
	26	Bayer Press Release, "Bayer and Regeneron Dose First Patient in Second Phase 3 Study for VEGF Trap-Eye in Wet Age-Related Macular Degeneration." May 8, 2008	Herewith
	27	Bayer Press Release, "VEGF Trap-Eye Shows Positive Results in Phase II Study in Patients with Diabetic Macular Edema" February 18, 2010	Herewith
	28	Bayer Press Release, "Bayer HealthCare and Regeneron Announce Encouraging 32-Week Follow Up Results From A Phase 2 Study of VEGF Trap-Eye in Age-Related Macular Degeneration" April 28, 2008	Herewith
	29	Bayer Press Release "Bayer HealthCare and Regeneron Announce VEGF Trap-Eye Achieved Durable Improvement in Vision Over 52 Weeks in a Phase 2 Study in Patients with Age-Related Macular Degeneration" August 19, 2008	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	30	BMJ Publishing Group Ltd., "Review: Ranibizumab (Lucentis) In Neovascular Age-Related Macular Degeneration: Evidence From Clinical Trials" British J. Ophthalmology (December 2020), https://bjo.bmj.com/content/94/1/2.altmetrics	Herewith
	31	Bontempo, "Preformulation Development of Parenteral Biopharmaceuticals" Drugs and the Pharmaceutical Sciences 85:91-108 (1997)	Herewith
	32	Bressler, N. M. and G. Treatment of Age-Related Macular Degeneration with Photodynamic Therapy Study, "Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: two-year results of 2 randomized clinical trials-tap report 2." Arch Ophthalmol 119(2): 198-207 (2001)	Herewith
	33	Brown & Regillo, "Anti-VEGF Agents in the Treatment of Neovascular Age-Related Macular Degeneration: Applying Clinical Trial Results to the Treatment of Everyday Patients" Am J. Ophthalmology 144: 627 (2007)	Herewith
	34	Chi *et al.*, "Physical Stability of Proteins in Aqueous Solution: Mechanism and Driving Forces in Nonnative Protein Aggregation" Pharmaceutical Research Vol. 20, No. 9, 1325-1336 (September 2003)	Herewith
	35	Ciulla & Rosenfeld, "Antivascular Endothelial Growth Factor Therapy For Neovascular Age-Related Macular Degeneration" Current Opinion Ophthalmology 20: 158 (2009)	Herewith
	36	Clinicaltrials.gov. I-SPY 2 TRIAL: Neoadjuvant and Personalized Adaptive Novel Agents to Treat Breast Cancer, Accessed 2010; http://clinical trials.gov/ct2/show/NCT01042379?term-NCT01042379&rank=1	Herewith
	37	CMS, Local Coverage Determination (LCD) for Ranibizumab (Lucentis) (L29266, First Coast Service Options, Inc June 14, 2011)	Herewith
	38	Controls in SCI experiments, RegenBase. Retrieved January 6, 2021, from http://regenbase.org/control-groups.html	Herewith
	39	Department of Health and Human Services, Office of Inspector General, "Questionable Billing for Medicare Ophthalmology Services" September 2015 OEI-04-12-00280	Herewith
	40	Drug Vehicle (Code C927), National Cancer Institute (NCI). Retrieved January 6, 2021, from https://ncithesaurus.nci.nih.gov/ncitbrowser/ConceptReport.jsp?dictionary =NCI_Thesaurus&code=C927&ns=ncit	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	41	EP 2 663 325 File History	Herewith
	42	Eylea® Prescribing Information, Revised 05/2019	Herewith
	43	Ferrara, N. & Kerbel, R., "Angiogenesis as a Therapeutic Target" Nature 438: 967 (2005)	Herewith
	44	Fraser *et al.*, "Single Injections of Vascular Endothelial Growth Factor Trap Block Ovulation in the Macaque and Produce a Prolonged, Dose-Related Suppression of Ovarian Function." J. Clin. Endocrinol & Metab. 90(2): 1114-1122 (February 2005)	Herewith
	45	Genentech, "FDA Approves Lucentis for the Treatment of Wet Age-Related Macular Degeneration," News Release dated June 30, 2006 (June 30, 2006)	Herewith
	46	Gupta, O. P., G. Shienbaum, A. H. Patel, C. Fecarotta, R. S. Kaiser and C. D. Regillo, "A treat and extend regimen using ranibizumab for neovascular age-related macular degeneration clinical and economic impact" Ophthalmology 117(11): 2134-2140 (2010)	Herewith
	47	Heier, "Intravitreal VEGF Trap for AMD: An Update" Retina Today 44 (October 2009)	Herewith
	48	Heier, J. S., P. A. Campochiaro, L. Yau, Z. Li, N. Saroj, R. G. Rubio and P. Lai "Ranibizumab for macular edema due to retinal vein occlusions: long-term follow-up in the HORIZON trial" Ophthalmology 119(4): 802-809 (2012)	Herewith
	49	HERCEPTIN® label	Herewith
	50	Holz *et al.*, "VEGF Trap-Eye for Macular Oedema Secondary to Central Retinal Vein Occlusion: 6-Month Results of the Phase III GALILEO Study" British J. Ophthalmology 97: 278 (2013)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	51	Ip, M. S., I. U. Scott, P. C. VanVeldhuisen, N. L. Oden, B. A. Blodi, M. Fisher, L. J. Singerman, M. Tolentino, C. K. Chan, V. H. Gonzalez and S. S. R. Group "A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with observation to treat vision loss associated with macular edema secondary to central retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 5" Arch Ophthalmol 127(9): 1101-1114 (2009)	Herewith
	52	Janeway *et al.*, "The structure of a typical antibody molecule" Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science (2001)	Herewith
	53	Keane *et al.*, "Effect of Ranibizumab Retreatment Frequency on Neurosensory Retinal Volume in Neovascular AMD" Retina 29: 592 (2009)	Herewith
	54	Kim *et al.*, "Potent VEGF Blockade Causes Regression of Coopted Vessels in a Model of Neuroblastoma" Proc. Nat'l Acad. Sci. 99: 11399 (2002)	Herewith
	55	LUCENTIS Approval (2006)	Herewith
	56	LUCENTIS® Label (14 pages)	Herewith
	57	LUCENTIS® Prescribing Information (2006)	Herewith
	58	Macular Photocoagulation Study, G., "Laser photocoagulation of subfoveal neovascular lesions in age-related macular degeneration. Results of a randomized clinical trial. Macular Photocoagulation Study Group" Arch Ophthalmol 109(9): 1220-1231 (1991)	Herewith
	59	Massin, "Anti-VEGF Therapy for Diabetic Macular Edema: An Update" Retina Today 54 (Sept./Oct. 2008)	Herewith
	60	Michels, S., P. J. Rosenfeld, C. A. Puliafito, E. N. Marcus and A. S. Venkatraman, "Systemic bevacizumab (Avastin) therapy for neovascular age-related macular degeneration twelve-week results of an uncontrolled open-label clinical study" Ophthalmology 112(6): 1035-1047 (2005)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	61	Mitchell *et al.*, "Ranibizumab (Lucentis) in Neovascular Age-Related Macular Degeneration: Evidence from Clinical Trials" Brit. J. Ophthalmology 94: 2 (2009)	Herewith
	62	Ni & Hui, "Emerging Pharmacologic Therapies for Wet Age-Related Macular Degeneration" Ophthalmologica 223: 401 (2009)	Herewith
	63	Parkins & Lashmar, "The formulation of biopharmaceutical products" Pharmaceutical Science & Technology Today Vol. 3, No. 4: 129-137 (April 4, 2000)	Herewith
	64	Phosphate buffer. Cold Spring Harbor Protocols 2006: pdb.rec8543 (2006)	Herewith
	65	Randolph & Jones, "Surfactant-Protein Interactions" Rational Design of Stable Protein Formulations pp. 159-175, Springer, Boston, MA (2002)	Herewith
	66	RAPTIVA® label	Herewith
	67	Regeneron Pharmaceuticals Inc. Regeneron Receives $20 Million Milestone Payment for Initiation of Phase 3 Study of VEGF Trap-Eye in Wet AMD. Media Release: 14 Aug 2007. Available from URL: http://www.regeneron.com	Herewith
	68	Regeneron Pharmaceuticals Inc. Regeneron Reports Fourth Quarter and Full Year 2004 Financial and Operating Results. Media Release: 22 Feb 2005. Available from URL: http://www.regeneron.com	Herewith
	69	Regeneron Pharmaceuticals Inc. Regeneron Reports Fourth Quarter and Full Year 2005 Financial and Operating Results. Media Release: 24 Feb 2006. Available from URL: http://regeneron.com	Herewith
	70	Regeneron Pharmaceuticals Inc. Regeneron Reports Positive Phase Data for the VEGF Trap in Age-Related Macular Degeneration; Preliminary Results Show Improvements in Vision and Reginal Swelling; VEGF Trap Was Well Tolerated at All Dose Levels. Media Release: 1 May 2006. Available from URL: http://www.regeneron.com	Herewith
	71	Regeneron SEC Form 10-Q (September 30, 2009)	Herewith
	72	Reichert, "Antibody-Based Therapeutics To Watch In 2011" MABS 3: 76 (2011)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	73	REMICADE® label	Herewith
	74	Retina Coding Q & A, Retinal Physician, 16: 18, 54 (July/August 2019)	Herewith
	75	Rogers *et al.*, "The prevalence of retinal vein occlusion: pooled data from population studies from the United States, Europe, Asia, and Australia" Ophthalmology 117(2): 313-319 e311 (2010)	Herewith
	76	Rosenfeld, P. J., A. A. Moshfeghi and C. A. Puliafito, "Optical coherence tomography findings after an intravitreal injection of bevacizumab (avastin) for neovascular age-related macular degeneration" Ophthalmic Surg Lasers Imaging 36(4): 331-335 (2005)	Herewith
	77	Rudge *et al.*, "VEGF Trap as a Novel Antiangiogenic Treatment Currently in Clinical Trials for Cancer and Eye Diseases, and VelociGene®-based Discovery of the Next Generation of Angiogenesis Targets," Cold Spring Harbor Symposia on Quantitative Biology 70: 411-418 (2005)	Herewith
	78	Schmidt-Erfurth "Current Concepts in the Management of Diabetic Macular Edema" Proceedings 7:52 (2010)	Herewith
	79	Scott *et al.*, "A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with standard care to treat vision loss associated with macular Edema secondary to branch retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 6" Arch Ophthalmol 127(9): 1115-1128 (2009)	Herewith
	80	SIMULECT® label	Herewith
	81	Spaide *et al.*, "Prospective Study of Intravitreal Ranibizumab as a Treatment for Decreased Visual Acuity Secondary to Central Retinal Vein Occlusion" Am J. Ophthalmology 147: 298 (2009)	Herewith
	82	Spielberg, L. & Leys, A., "Intravitreal Bevacizumab for Myopic Choroidal Neovascularization: Short-Term and 1-Year Results" Bulletin Societe Belge D'Ophtalmologie 312: 17 (2009)	Herewith
	83	Steinbrook, "The Price of Sight — Ranibizumab, Bevacizumab, and the Treatment of Macular Degeneration" N. Eng. J. Med. 355:1409 (2006)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	Regeneron Pharmaceuticals, Inc.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	84	The Branch Vein Occlusion Study, G., "Argon laser photocoagulation for macular edema in branch vein occlusion" Am J Ophthalmol 98(3): 271-282 (1984)	Herewith
	85	The Central Vein Occlusion Study, G., "Evaluation of grid pattern photocoagulation for macular edema in central vein occlusion. The Central Vein Occlusion Study Group M report" Ophthalmology 102(10): 1425-1433 (1995)	Herewith
	86	U.S. DEP'T HEALTH & HUMAN SERVS., NAT'L INST. HEALTH, NAT'L EYE INST., "Age-Related Macular Degeneration: What You Should Know" (Sept. 2015) https://www.nei.nih.gov/sites/default/files/healthpdfs/WYSK_AMD_English_Sept2015_PRINT.pdf	Herewith
	87	U.S. DEP'T HEALTH & HUMAN SERVS., NAT'L INST. HEALTH, NAT'L EYE INST., "Diabetic Retinopathy: What You Should Know" (Sept. 2015), https://www.nei.nih.gov/sites/default/files/2019-06/Diabetic-Retinopathy-What-You-Should-Know-508.pdf	Herewith
	88	U.S. Department of Health and Human Services Food and Drug Administration, "Guidance for industry Q1A(R2) stability testing of new drug substances and products" Rockville, MD (November 2003)	Herewith
	89	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) (VIEW1), NCT00509795, ClinicalTrials.gov (Apr. 28, 2009), https://clinicaltrials.gov/ct2/show/NCT00509795 ("NCT-795")	Herewith
	90	Wall Street Journal, "Genentech's Big Drug for Eyes Faces a Rival" (2007)	Herewith
	91	Wulff *et al.,* "Prevention of Thecal Angiogenesis, Antral Follicular Growth, and Ovulation in the Primate by Treatment with Vascular Endothelial Growth Factor Trap R1R2" Endocrinology 143(7): 2797-2807 (July 2002)	Herewith
	92	XOLAIR® label	Herewith
	93	Zarbin & Rosenfeld, "Pathway-Based Therapies for Age-Related Macular Degeneration: An Integrated Survey of Emerging Treatment Alternatives" Retina 30: 1350 (2010)	Herewith

EXAMINER	DATE CONSIDERED
/JON M LOCKARD/	09/29/2022

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
Approved for use through 01/31/2018. OMB 0651-0035
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

POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date
17/072,417	October 16, 2020

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 96387

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

☑ The address associated with the above-mentioned Customer Number

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

Regeneron Pharmaceuticals, Inc.

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature	/Frank R. Cottingham/	Date (Optional)	
Name	Frank R. Cottingham		
Title	Vice President Intellectual Property, Regeneron Pharmaceuticals, Inc.		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Acknowledgement Receipt

EFS ID:	46842937
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Savanna Fuentes
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	18-OCT-2022
Filing Date:	16-OCT-2020
Time Stamp:	16:15:51
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	Assignee showing of ownership per 37 CFR 3.73	REGN-008CIPCON6_2022-10-18_373c.pdf	32275 1f10347c352156d5bf89612db2a85e5e3b7b2425	no	2

Warnings:

Information:					
2	Power of Attorney	0725US07_POA.pdf	163537 587c15449629b5ed4d56fa28826697309b4122a6	no	1
Warnings:					
Information:					
		Total Files Size (in bytes):	195812		

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.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: **Regeneron Pharmaceuticals, Inc.**

Application No./Patent No.: **17/072,417** Filed/Issue Date: **October 16, 2020**

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 (choose **one** of options 1, 2, 3 or 4 below):

1. ☒ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

   ☐ The extent (by percentage) of its ownership interest is _____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.,* bankruptcy, probate), or an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel **054907**, Frame **0382**, 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.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Karl Bozicevic/	**October 18, 2022**
Signature	Date
Karl Bozicevic	**28,807**
Printed or Typed Name	Title or Registration Number

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

U.S. PATENT DOCUMENTS					
		DOCUMENT NUMBER	DATE	NAME	REFERENCE PROVIDED*
	1.	US 7,300,563 B2	2007-11-27	Diaddario, Jr.	not required per 69 Fed. Reg. 56481
	2.	US 7,300,653 B2	2007-11-27	Wiegand *et al.*	not required per 69 Fed. Reg. 56481
	3.	US 7,608,261 B2	2009-10-27	Furfine *et al.*	not required per 69 Fed. Reg. 56481
	4.	US 2010/0160,233 A1	2010-06-24	Bissery *et al.*	not required per 69 Fed. Reg. 56481
	5.	US 7,972,598 B2	2011-07-05	Daly *et al.*	not required per 69 Fed. Reg. 56481
	6.	US 8,029,791 B2	2011-10-04	Papadopoulos *et al.*	not required per 69 Fed. Reg. 56481
	7.	US 8,343,737 B2	2013-01-01	Papadopoulos *et al.*	not required per 69 Fed. Reg. 56481
	8.	US 8,647,842 B2	2014-02-11	Papadopoulos *et al.*	not required per 69 Fed. Reg. 56481
	9.	US 10,857,205 B2	2020-12-08	Yancopoulos	not required per 69 Fed. Reg. 56481
	10.	US 10,888,601 B2	2021-01-12	Yancopoulos	not required per 69 Fed. Reg. 56481
	11.	US 11,066,458 B2	2021-07-20	Furfine *et al.*	not required per 69 Fed. Reg. 56481
	12.	US 11,084,865 B2	2021-08-10	Furfine *et al.*	not required per 69 Fed. Reg. 56481
	13.	US 11,253,572 B2	2022-02-22	Yancopoulos	not required per 69 Fed. Reg. 56481

FOREIGN PATENT DOCUMENTS						
		DOCUMENT NUMBER	DATE	COUNTRY	TRANSLATION	REFERENCE PROVIDED*
	14.	EP 3222285 A1	2017-09-27	EPO	N/A	Herewith

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	15.	Abraham *et al.*, "Randomized, Double-Masked, Sham-Controlled Trial of Ranibizumab for Neovascular Age-Related Macular Degeneration: PIER Study Year 2," *Am. J. Ophthalmology*, 150(3), pp. 315-324.e1 (September 2010)	Herewith
	16.	Adamis, "Ocular Angiogenesis: Vascular Endothelial Growth Factor and Other Factors," in *Retinal Pharmacotherapy 23*, Nguyen *et al.*, eds., (2010)	Herewith
	17.	American Academy of Ophthalmology, "Anti-VEGF Treatments," https://www.aao.org/eye-health/drugs/anti-vegf-treatments (accessed November 8, 2021)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	18.	American Academy of Ophthalmology, "Bevacizumab," https://eyewiki.aao.org/Bevacizumab (accessed November 2, 2021)	Herewith
	19.	American Academy of Ophthalmology, "Ophthalmology Subspecialists," June 6, 2016, https://www.aao.org/eye-health/tips-prevention/ophthalmology-subspecialists (accessed September 26, 2022)	Herewith
	20.	American Academy of Ophthalmology, "Retinal Vasculitis," https://eyewiki.aao.org/Retinal_Vasculitis (accessed January 13, 2022)	Herewith
	21.	American Academy of Ophthalmology, "What is Avastin," https://www.aao.org/eye-health/drugs/avastin (accessed November 9, 2021)	Herewith
	22.	American Academy of Ophthalmology, "What is Eylea," https://www.aao.org/eye-health/drugs/what-is-eylea (accessed November 9, 2021)	Herewith
	23.	American Academy of Ophthalmology, "What is Lucentis," https://www.aao.org/eye-health/drugs/lucentis (accessed November 9, 2021)	Herewith
	24.	American Society of Retina Specialists, "About Us," https://www.asrs.org/about (accessed December 6, 2021)	Herewith
	25.	American Society of Retina Specialists, "Age-Related Macular Degeneration," https://www.asrs.org/patients/retinal-diseases/2/agerelated-macular-degeneration (accessed December 30, 2021)	Herewith
	26.	American Society of Retina Specialists, "Branch Retinal Vein Occlusion," https://www.asrs.org/patients/retinal-diseases/24/branch-retinal-vein-occlusion (accessed December 30, 2021)	Herewith
	27.	American Society of Retina Specialists, "Central Retinal Vein Occlusion," https://www.asrs.org/patients/retinal-diseases/22/central-retinal-vein-occlusion (accessed December 30, 2021)	Herewith
	28.	American Society of Retina Specialists, "Diabetic Retinopathy," https://www.asrs.org/patients/retinal-diseases/3/diabetic-retinopathy (accessed December 30, 2021)	Herewith
	29.	American Speech-Language-Hearing Association, "Calculating Medicare Fee Schedule Rates," https://www.asha.org/practice/reimbursement/medicare/calculating-medicare-fee-schedule-rates/ (accessed November 22, 2021)	Herewith
	30.	*Amgen v. F. Hoffman-La Roche, Ltd.*, Case No. 05-cv-12237 (D. Mass.), ECF 610-3, Declaration of Alexander M. Klibanov, Ph.D. in Support of Defendants' Opposition to Amgen's Motion for Summary Judgment of Infringement of '422 Claim 1, '933 Claim 3, and '698 Claim 6 (June 28, 2007), cited in Deposition of Dr. Alexander M. Klibanov, Ph.D., on March 24, 2022	Herewith
	31.	Amgen, "Fusion Protein," https://www.amgen.com/stories/2018/08/the-shape-of-drugs-to-come/fusion-protein (accessed January 7, 2022)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	32.	Amino acid sequence alignment of SEQ ID NO:2 of the '338 and '069 patents with aflibercept amino acid sequence from WHO 2006, SEQ ID NO:16 of the '758 patent, and SEQ ID NO:16 of the '959 patent, submitted on May 27, 2022, in IPR2021-00881 as Exhibit 1122	Herewith
	33.	Amino acid sequence alignment of SEQ ID NO:2 of the '338 patent with SEQ ID NO:16 of the '758 patent and SEQ ID NO:4 of Dix, submitted in IPR2022-00881 as Exhibit 1093	Herewith
	34.	Amino acid sequence alignment of SEQ ID NO:2 of the '338 patent, aflibercept amino acid sequence from WHO 2006, and SEQ ID NO:2 of the '173 patent, cited in Deposition of Dr. Alexander M. Klibanov, Ph.D., on March 24, 2022, submitted in IPR2021-00881 as Exhibit 1117	Herewith
	35.	Amino acid sequence alignment of SEQ ID NO:2 of the '681 and '601 patents with aflibercept amino acid sequence from WHO 2006, SEQ ID NO:16 of the '758 patent, and SEQ ID NO:16 of the '959 patent, submitted in IPR2022-01226 as Exhibit 1087	Herewith
	36.	Amino acid sequence alignment of SEQ ID NO:2 of the '681 and '601 patents with SEQ ID NO:16 of the '758 patent and SEQ ID NO:2 of the '173 patent, submitted in IPR2022-01226 as Exhibit 1092	Herewith
	37.	Annotated version of '338 patent claim 1, cited in Deposition of Dr. Diana V. Do, M.D., on April 21, 2022	Herewith
	38.	ASRS Clinical Updates, "ASRS Fights Novitas [sic] Decision to Interpret Eylea Usage More Frequently than q8 as 'Off Label'," (May 24, 2016) (accessed April 7, 2022), cited in Deposition of Dr. David M. Brown, M.D., on April 26, 2022	Herewith
	39.	Avastin Label (revised 2004), https://www.accessdata.fda.gov/drugsatfda_docs/label/2004/125085lbl.pdf (accessed September 26, 2022)	Herewith
	40.	BasePair Biotechnologies, "What is an Aptamer? – Aptamers and SELEX," https://www.basepairbio.com/what-is-an-aptamer/ (accessed December 30, 2021)	Herewith
	41.	Batta *et al.*, "Trends in FDA Drug Approvals Over Last 2 Decades: An Observational Study," *J. FAMILY MEDICINE & PRIMARY CARE*, 9, pp. 105-114 (2020)	Herewith
	42.	Bausch and Lomb, "Help Your Patients Obtain Access to Visudyne," https://www.bauschretinarx.com/visudyne/ecp/ordering/ (accessed January 12, 2022)	Herewith
	43.	Bausch and Lomb, "Visudyne," https://www.bauschretinarx.com/visudyne/ecp/about/ (accessed December 2, 2021)	Herewith
	44.	Bausch Health Companies, Form 10-K, 2020	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	45.	BCBS Florida, "Vascular Endothelial Growth Factor Inhibitors for Ocular Neovascularization," revised April 1, 2022	Herewith
	46.	Beovu Label (revised June 2020), https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/761125s004lbl.pdf (accessed September 26, 2022)	Herewith
	47.	Beovu Label (revised October 2019), https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/761125s000lbl.pdf (accessed September 26, 2022)	Herewith
	48.	Bhisitkul *et al.*, "Alternative anti-VEGF treatment regimens in exudative age-related macular degeneration," *Expert Rev. Ophthalmol.*, 5(6) (January 2010)	Herewith
	49.	BIOSPACE, "Bayer HealthCare AG and Regeneron Pharmaceuticals, Inc. to Collaborate on VEGF Trap for the Treatment Of Eye Diseases; Regeneron Retains U.S. Commercialization Rights, Receives $75 Million Upfront, and Eligible for up to $245 Million of Milestone Payments," (October 19, 2006), https://www.biospace.com/article/releases/bayer-healthcare-ag-and-regeneron-pharmaceuticals-inc-to-collaborate-on-vegf-trap-for-the-treatment-of-eye-diseases-b-regeneron-b-retains-u-s-c/ (accessed September 26, 2022)	Herewith
	50.	Bork *et al.*, "Increasing the Sialylation of Therapeutic Glycoproteins: The Potential of the Sialic Acid Biosynthetic Pathway," *J. Pharm. Sci.*, 98(10), pp. 3499-3508 (October 2009)	Herewith
	51.	Bright Focus Foundation, "Age-Related Macular Degeneration: Facts & Figures," https://www.brightfocus.org/macular/article/age-related-macular-facts-figures (accessed November 5, 2021)	Herewith
	52.	Brown *et al.*, "Intravitreal Aflibercept Injection for Macular Edema Secondary to Central Retinal Vein Occlusion: 1-Year Results from the Phase 3 COPERNICUS Study", *Am. J. Ophthalmol.*, 155, pp. 329-437 (March 2013)	Herewith
	53.	Brown *et al.*, "Ranibizumab Versus Verteporfin Photodynamic Therapy for Neovascular Age-Related Macular Degeneration: Two-Year Results of the ANCHOR Study," *Ophthalmology*, 116(1), pp. 57-65.e5 (January 2009)	Herewith
	54.	Calculator.net, "Sample Size Calculator," https://www.calculator.net/sample-size-calculator.html?type=2&cl2=95&ss2=200&pc2=50&ps2=3000&x=68&y=18#findci (accessed January 25, 2022)	Herewith
	55.	Campochiaro *et al.*, "Antagonism of Vascular Endothelial Growth Factor for Macular Edema Caused by Retinal Vein Occlusions: Two-Year Outcomes," *Ophthalmology*, 117(12), pp. 2387-2394.e5 (December 2010) (online publication)	Herewith
	56.	Cantu *et al.*, "Thioesterases: A New Perspective Based on Their Primary and Tertiary Structures," *Protein Science*, 19(17), pp. 1281-1295 (July 2010)	Herewith
	57.	CAS registry for No. 862111-32-8, cited in Deposition of Dr. Alexander M. Klibanov, Ph.D., on March 24, 2022	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	58.	Center for Drug Evaluation and Research, Approved Labeling for BLA Application No. 125156 (Lucentis) (2006)	Herewith
	59.	Center for Drug Evaluation and Research, Medical Review for BLA Application No. 125387 (November 18, 2011)	Herewith
	60.	Center for Drug Evaluation and Research, Statistical Review for BLA Application No. 125387 (November 18, 2011)	Herewith
	61.	Centers for Disease Control and Prevention, "Vision Loss: A Public Health Problem," https://www.cdc.gov/visionhealth/basic_information/vision_loss.htm (accessed June 12, 2020)	Herewith
	62.	Centers for Medicare & Medicaid Services, "Medicare Physician & Other Practitioners - by Provider and Service," https://data.cms.gov/provider-summary-by-type-of-service/medicare-physician-other-practitioners/medicare-physician-other-practitioners-by-provider-and-service (accessed November 19, 2021)	Herewith
	63.	Centers for Medicare & Medicaid Services, "Payment Allowance Limits for Medicare Part B Drugs: Effective October 1, 2012, through December 31, 2012," (October 2012), https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Part-B-Drugs/McrPartBDrugAvgSalesPrice/2012ASPFiles (accessed September 26, 2022)	Herewith
	64.	Centers for Medicare & Medicaid Services, "Payment Allowance Limits for Medicare Part B Drugs: Effective October 1, 2013, through December 31, 2013," (October 2013), https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Part-B-Drugs/McrPartBDrugAvgSalesPrice/2013ASPFiles (accessed September 26, 2022)	Herewith
	65.	Centers for Medicare & Medicaid Services, "Payment Allowance Limits for Medicare Part B Drugs: Effective October 1, 2014, through December 31, 2014," (October 2014), https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Part-B-Drugs/McrPartBDrugAvgSalesPrice/2014ASPFiles (accessed September 26, 2022)	Herewith
	66.	Centers for Medicare & Medicaid Services, "Payment Allowance Limits for Medicare Part B Drugs: Effective October 1, 2015, through December 31, 2015," (October 2015), https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Part-B-Drugs/McrPartBDrugAvgSalesPrice/2015ASPFiles (accessed September 26, 2022)	Herewith
	67.	Centers for Medicare & Medicaid Services, "Payment Allowance Limits for Medicare Part B Drugs: Effective October 1, 2016, through December 31, 2016," (October 2016), https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Part-B-Drugs/McrPartBDrugAvgSalesPrice/2016ASPFiles (accessed September 26, 2022)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	68.	Centers for Medicare & Medicaid Services, "Payment Allowance Limits for Medicare Part B Drugs: Effective October 1, 2017, through December 31, 2017," (October 2017), https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Part-B-Drugs/McrPartBDrugAvgSalesPrice/2017ASPFiles (accessed September 26, 2022)	Herewith
	69.	Centers for Medicare & Medicaid Services, "Payment Allowance Limits for Medicare Part B Drugs: Effective October 1, 2018, through December 31, 2018," (October 2018), https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Part-B-Drugs/McrPartBDrugAvgSalesPrice/2018ASPFiles (accessed September 26, 2022)	Herewith
	70.	Centers for Medicare & Medicaid Services, "Payment Allowance Limits for Medicare Part B Drugs: Effective October 1, 2019, through December 31, 2019," (October 2019), https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Part-B-Drugs/McrPartBDrugAvgSalesPrice/2019ASPFiles (accessed September 26, 2022)	Herewith
	71.	Centers for Medicare & Medicaid Services, "Payment Allowance Limits for Medicare Part B Drugs: Effective October 1, 2020, through December 31, 2020," (October 2020), https://www.cms.gov/medicare/medicare-part-b-drug-average-sales-price/2020-asp-drug-pricing-files (accessed September 26, 2022)	Herewith
	72.	Centers for Medicare & Medicaid Services, "Payment Allowance Limits for Medicare Part B Drugs: Effective October 1, 2021, through December 31, 2021," (October 2021), https://www.cms.gov/medicare/medicare-part-b-drug-average-sales-price/2021-asp-drug-pricing-files (accessed September 26, 2022)	Herewith
	73.	Centers for Medicare & Medicaid Services, "Physician Fee Schedule," https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Payment/PhysicianFeeSched (accessed November 22, 2021)	Herewith
	74.	Centers for Medicare & Medicare Services, "2021 ASP Drug Pricing Files," https://www.cms.gov/medicare/medicare-part-b-drug-average-sales-price/2021-asp-drug-pricing-files (accessed November 22, 2021)	Herewith
	75.	Centers for Medicare & Medicare Services, "Medicare Part B Drug Average Sales Price," https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Part-B-Drugs/McrPartBDrugAvgSalesPrice (accessed December 8, 2021)	Herewith
	76.	Chen *et al.*, "Carboxylic ester hydrolases: Classification and database derived from their primary, secondary, and tertiary structures," *Protein Science*, 25(11), pp. 1942-1953 (November 2016)	Herewith
	77.	Christensen, "Methodology of Superiority vs. Equivalence Trials and Non-Inferiority Trials," *J. HEPATOLOGY*, 46(5), pp. 947-954 (May 2007) (online publication)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	78.	Clark *et al.*, "Treatment Paradigms in AMD Management: Assessing Consistent Long-Term Dosing," *RETINA TODAY SUPP.*, pp. 1-16 (September 2017), cited in Deposition of Dr. David M. Brown, M.D., on April 26, 2022	Herewith
	79.	ClinicalTrials.gov Archive, History of Changes for Study: NCT00473330, "A Study of Ranibizumab Injection in Subjects With Clinically Significant Macular Edema (ME) With Center Involvement Secondary to Diabetes Mellitus (RISE)," Version 13, dated March 21, 2017, submitted in IPR2021-00881 as Exhibit 2122	Herewith
	80.	ClinicalTrials.gov Archive, History of Changes for Study: NCT00473382, "A Study of Ranibizumab Injection in Subjects With Clinically Significant Macular Edema (ME) With Center Involvement Secondary to Diabetes Mellitus (RIDE)," Version 13, dated March 21, 2017, submitted in IPR2021-00881 as Exhibit 2123	Herewith
	81.	ClinicalTrials.gov Archive, History of Changes for Study: NCT00485836, "A Study of the Efficacy and Safety of Ranibizumab Injection in Patients With Macular Edema Secondary to Central Retinal Vein Occlusion (CRUISE)," Version 10, dated June 29, 2017, submitted in IPR2021-00881 as Exhibit 2125	Herewith
	82.	ClinicalTrials.gov Archive, History of Changes for Study: NCT00486018, "A Study of the Efficacy and Safety of Ranibizumab Injection in Patients With Macular Edema Secondary to Branch Retinal Vein Occlusion (BRAVO)," Version 12, dated April 4, 2017, submitted in IPR2021-00881 as Exhibit 2124	Herewith
	83.	ClinicalTrials.gov Archive, History of Changes for Study: NCT00519285, "Aflibercept in Combination With Docetaxel in Metastatic Androgen Independent Prostate Cancer (VENICE)," Version 01, dated August 21, 2007, submitted in IPR2021-00881 as Exhibit 2078	Herewith
	84.	ClinicalTrials.gov Archive, History of Changes for Study: NCT00644124, "Aflibercept and Standard Chemotherapy (R-CHOP) in First Line of Non Hodgkin B-Cell Lymphoma," Version 01, dated March 21, 2008, submitted in IPR2021-00881 as Exhibit 2079	Herewith
	85.	ClinicalTrials.gov Archive, History of Changes for Study: NCT00794417, "A Study of Aflibercept Administered in Combination With Pemetrexed and Cisplatin in Patients With Advanced Carcinoma," Version 01, dated November 19, 2008, submitted in IPR2021-00881 as Exhibit 2053	Herewith
	86.	ClinicalTrials.gov Archive, History of Changes for Study: NCT01148615, "A Study of Intravenous Aflibercept With Docetaxel in Chinese Patients With Solid Tumors," Version 01, dated June 21, 2010, submitted in IPR2021-00881 as Exhibit 2054	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	87.	ClinicalTrials.gov Archive, History of Changes for Study: NCT01486771, "Macugen for Proliferative Diabetic Retinopathy Study With Extended Dosing (M-PDRS ED)," Version 01, dated December 5, 2011, submitted in IPR2021-00881 as Exhibit 2109	Herewith
	88.	ClinicalTrials.gov Archive, History of Changes for Study: NCT01940900, "A Phase 3 Safety and Efficacy Study of Fovista (E10030) Intravitreous Administration in Combination With Lucentis Compared to Lucentis Monotherapy," Version 21, dated August 13, 2018, submitted in IPR2021-00881 as Exhibit 2025	Herewith
	89.	ClinicalTrials.gov Archive, History of Changes for Study: NCT01944839, "A Phase 3 Safety and Efficacy Study of Fovista (E10030) Intravitreous Administration in Combination With Lucentis Compared to Lucentis Monotherapy," Version 27, dated August 8, 2018, submitted in IPR2021-00881 as Exhibit 2024	Herewith
	90.	ClinicalTrials.gov Archive, History of Changes for Study: NCT02247479, "A Study Investigating the Efficacy and Safety of Lampalizumab Intravitreal Injections in Participants With Geographic Atrophy Secondary to Age-Related Macular Degeneration (CHROMA)," Version 60, dated June 17, 2019, submitted in IPR2021-00881 as Exhibit 2021	Herewith
	91.	ClinicalTrials.gov Archive, History of Changes for Study: NCT02247531, "A Study Investigating the Safety and Efficacy of Lampalizumab Intravitreal Injections in Participants With Geographic Atrophy Secondary to Age-Related Macular Degeneration (SPECTRI)," Version 60, dated October 14, 2019, submitted in IPR2021-00881 as Exhibit 2020	Herewith
	92.	ClinicalTrials.gov Archive, History of Changes for Study: NCT03577899, "Efficacy and Safety Trial of Conbercept Intravitreal Injection for Neovascular AMD(PANDA-1)," Version 06, dated June 23, 2021, submitted in IPR2021-00881 as Exhibit 2023	Herewith
	93.	ClinicalTrials.gov Archive, History of Changes for Study: NCT03630952, "Efficacy and Safety Trial of Conbercept Intravitreal Injection for Neovascular AMD(PANDA-2)," Version 07, dated June 22, 2021, submitted in IPR2021-00881 as Exhibit 2022	Herewith
	94.	ClinicalTrials.gov, "1997: Congress Passes Law (FDAMA) Requiring Trial Registration," https://clinicaltrials.gov/ct2/about-site/history (accessed April 26, 2021)	Herewith
	95.	ClinicalTrials.gov, "What Is ClinicalTrials.gov?" https://www.clinicaltrials.gov/ct2/about-site/background (accessed January 20, 2021)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	96.	CloudResearch, "Determining Sample Size: How Many Survey Participants Do You Need?" https://www.cloudresearch.com/resources/guides/statistical-significance/determine-sample-size/ (accessed January 25, 2022)	Herewith
	97.	CMS.gov Medicare Coverage Database, "Billing and Coding: Aflibercept (Eylea)," https://www.cms.gov/medicare-coverage-database/view/article.aspx?articleid=53387&ver=28&keyword=&keywordType=starts&areaId=all&docType=6,3,5,1,F,P&contractOption=all&hcpcsOption=code&hcpcsStartCode=J0178&hcpcsEndCode=J0178&sortBy=title&bc=1 (accessed April 22, 2021)	Herewith
	98.	Cobo *et al.*, "The Clearance of Intravitreal Gentamicin," *Am. J. Ophthalmology*, 92(1), pp. 59-62 (1981)	Herewith
	99.	Complaint, *Horizon Healthcare Servs., Inc. v. Regeneron Pharms., Inc.*, No. 1:22-cv-10493-FDS (D. Mass. April 4, 2022), ECF Nos. 1 – 1-18	Herewith
	100.	Complaint, *United States v. Regeneron Pharms., Inc.,* No. 1:20-cv-11217-FDS (D. Mass. June 24, 2020), ECF Nos. 1 — 1-39	Herewith
	101.	Corporate Finance Institute, "SEC Filings - Requirements for Public Companies & Where to Find Them," https://corporatefinanceinstitute.com/resources/data/public-filings/sec-filings/ (accessed January 20, 2021)	Herewith
	102.	Cousins, "Controversies in the Long-term Management of Neovascular AMD: The Role of Imaging in Clinical Decision Making," *Retinal Physician* (January 1, 2010), https://www.retinalphysician.com/issues/2010/jan-feb/controversies-in-the-long-term-management-of-neova (accessed September 26, 2022)	Herewith
	103.	Cruz, "PIER Data Suggest a Need for Tailored Injection Schedule," *Ocular Surgery News*, (September 1, 2006), https://www.healio.com/news/ophthalmology/20120331/pier-data-suggest-a-need-for-tailored-injection-schedule (accessed February 10, 2022)	Herewith
	104.	Dadgostar *et al.*, "Evaluation of Injection Frequency and Visual Acuity Outcomes for Ranibizumab Monotherapy in Exudative Age-related Macular Degeneration," *Ophthalmology*, 116, pp. 1740-1747 (2009)	Herewith
	105.	Declaration of Doris Weber dated March 7, 2022, in IPR2021-00881	Herewith
	106.	Demarest *et al.*, "Optimization of the Antibody C_H3 Domain by Residue Frequency Analysis of IgG Sequences," *J. Mol. Biol.*, 335(1), pp. 41-48 (January 2004)	Herewith
	107.	Do *et al.*, "Pharmacokinetic Study of Intravitreal Aflibercept In Humans with Neovascular Age-Related Macular Degeneration," *RETINA*, 00, pp. 1-5 (2019), also available as *RETINA*, 40(4), pp. 643-647 (April 2020)	Herewith
	108.	Donohue *et al.*, "A Decade of Direct-to-Consumer Advertising of Prescription Drugs," *The New England Journal of Medicine*, 35(7), pp. 673-681 (August 2007)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	109.	Donohue *et al.*, "Effect of Direct-to-Consumer Advertising on Medication Choice: The Case of Antidepressants," Journal of Public Policy & Marketing, 23(2), pp. 115-127 (September 2004)	Herewith
	110.	Dreyfuss *et al.*, "Ocular Angiogenesis," *Journal of Ophthalmology*, 2015, pp. Article ID 892043 (September 2015)	Herewith
	111.	Drugs.com, "Eylea FDA Approval History," https://www.drugs.com/history/eylea.html (accessed November 16, 2021)	Herewith
	112.	Drugs.com, "FDA Approves Eylea for Wet Age-Related Macular Degeneration," (November 18, 2011), https://www.drugs.com/newdrugs/fda-approves-eylea-wet-age-related-macular-degeneration-2955.html (accessed February 4, 2022)	Herewith
	113.	Duncan *et al.*, "Inhibition of Vascular Endothelial Growth Factor in the Primate Ovary Up-Regulates Hypoxia-Inducible Factor-1α in the Follicle and Corpus Luteum," *ENDOCRINOLOGY*, 149, pp. 3313-3320 (April 2008) (online publication), cited in Deposition of Dr. Alexander M. Klibanov, Ph.D., on March 24, 2022	Herewith
	114.	Elvidge, "Opthotech's Fovista crashes out in wet AMD," *BIOPHARMADIVE* (August 14, 2017), available at https://www.biopharmadive.com/news/opthotech-fovista-phase-3-failure-setback-novartis/449248/ (accessed August 2, 2021)	Herewith
	115.	Elyasi *et al.*, "Diabetic Macular Edema: Diagnosis and Management," EyeNet Magazine, May 2021: 35-37 (May 2021)	Herewith
	116.	EP Patent Application No. 3 222 285 File History	Herewith
	117.	Ex. (a)(1)(a) to Tender Offer Statement to Momenta, filed with SEC on September 2, 2020	Herewith
	118.	Excerpts from J.M. Berg *et al.*, Biochemistry (5th Ed. 2002)	Herewith
	119.	Expert Declaration of Angelo P. Tanna, M.D., dated September 6, 2022, in IPR2022-01524	Herewith
	120.	Expert Declaration of David M. Brown, M.D., dated February 10, 2022, in IPR2021-00880 and IPR2021-00881	Herewith
	121.	Expert Declaration of Dr. Alexander M. Klibanov, Ph.D., dated February 8, 2022, in IPR2021-00880 and IPR2021-00881 - [[REDACTED]]	Herewith
	122.	Expert Declaration of Dr. Diana V. Do, M.D., dated February 10, 2022, in IPR2021-00881	Herewith
	123.	Expert Declaration of Dr. Lucian V. Del Priore, M.D., Ph.D., dated February 9, 2022, in IPR2021-00880 and IPR2021-00881 - [[REDACTED]]	Herewith
	124.	Expert Declaration of Dr. Thomas A. Albini in Support of Petition for Inter Partes Review of Patent No. 10,130,681 B2, dated June 30, 2022, in IPR2022-01225	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	125.	Expert Declaration of Dr. Thomas A. Albini in Support of Petition for Inter Partes Review of Patent No. 10,888,601 B2, dated June 30, 2022, in IPR2022-01226	Herewith
	126.	Expert Declaration of Dr. Thomas A. Albini in Support of Petitioner's Reply, dated May 27, 2022, in IPR2021-00881	Herewith
	127.	Expert Declaration of Ivan T. Hofmann Support of Petitioner's Reply, dated May 27, 2022, in IPR2021-00881 - [[REDACTED]]	Herewith
	128.	Expert Declaration of Mary Gerritsen, Ph.D. in Support of Petition for Inter Partes Review of U.S. Patent No. 10,130,681 B2, dated June 30, 2022, in IPR2022-01225	Herewith
	129.	Expert Declaration of Mary Gerritsen, Ph.D. in Support of Petition for Inter Partes Review of U.S. Patent No. 10,888,601 B2, dated June 30, 2022, in IPR2022-01226	Herewith
	130.	Expert Declaration of Mary Gerritsen, Ph.D. in Support of Petitioner's Reply, dated May 26, 2022, in IPR2021-00881	Herewith
	131.	Expert Declaration of Richard Manning, Ph.D., dated February 11, 2022, in IPR2021-00881 - [[REDACTED]]	Herewith
	132.	Eye Care Surgery Center, "Macular Degeneration," https://www.eyecaresurgerycenterbr.com/diabetes-retina/macular-degeneration/ (accessed November 18, 2021)	Herewith
	133.	EyeGuru.org, "Intravitreal Injection Standard Dosing Table," https://eyeguru.org/blog/intravitreal-injection-dosing/ (accessed December 6, 2021)	Herewith
	134.	Eylea Label (revised March 2021), https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/125387s069lbl.pdf (accessed September 26, 2022)	Herewith
	135.	Eylea Label (revised May 2016), https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/125387s051lbl.pdf (accessed September 26, 2022)	Herewith
	136.	Eylea Label (revised October 2014), https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/125387s043lbl.pdf (accessed September 26, 2022)	Herewith
	137.	Eylea Label (revised September 2012), https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125387s004lbl.pdf (accessed September 26, 2022)	Herewith
	138.	Eylea, "Wet AMD: Dosing Flexibility," https://hcp.eylea.us/about/wet-amd-dosing/ (accessed January 5, 2022)	Herewith
	139.	Eylea Approval Letter (November 18, 2011)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	140.	Fauser *et al.*, "Suppression of Intraocular Vascular Endothelial Growth Factor During Aflibercept Treatment of Age-Related Macular Degeneration," *Am. J. Ophthalmology*, 158, pp. 532-536 (2014)	Herewith
	141.	FDA Center for Drug Evaluation and Research, "Application Number: 125387Orig1s000 [EYLEA] Summary Review," https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/125387Orig1s000SumR.pdf (accessed May 20, 2022)	Herewith
	142.	FDA, "Drugs@FDA: FDA-Approved Drugs, BLA 125387," https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=125387 (accessed May 18, 2022)	Herewith
	143.	FDA, "22 Case Studies Where Phase 2 and Phase 3 Trials Had Divergent Results" (January 2017), submitted in IPR2021-00881 as Exhibit 1146	Herewith
	144.	FDA, "Guidance for Industry: Expedited Programs for Serious Conditions—Drugs and Biologics" (May 2014), https://www.fda.gov/media/86377/download (accessed September 26, 2022)	Herewith
	145.	FDA, "Macugen Drug Approval Package Page," March 23, 2005, https://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-756_Macugen.cfm (accessed January 12, 2022)	Herewith
	146.	FDA, "Non-Inferiority Clinical Trials to Establish Effectiveness: Guidance for Industry" (November 2016), submitted in IPR2021-00881 as Exhibit 2097	Herewith
	147.	FDA, "Purple Book Database of Licensed Biological Products," https://purplebooksearch.fda.gov/patent-list (accessed May 13, 2022)	Herewith
	148.	Fernández-Ferreiro *et al.*, "Preclinical PET Study of Intravitreal Injections," *Investigative Ophthalmology & Visual Science*, 58(7), pp. 2843-2851 (June 2017)	Herewith
	149.	Ferrara *et al.*, "Development of ranibizumab, an anti-vascular endothelial growth factor antigen binding fragment, as therapy for neovascular age-related macular degeneration," *Retina*, 26(8), pp. 859-870 (October 2006)	Herewith
	150.	FiercePharma, "Beovu, Novartis," (October 25, 2021), https://www.fiercepharma.com/special-report/beovu-novartis-top-10-drug-launch-disasters (accessed December 30, 2021)	Herewith
	151.	FiercePharma, "Novartis' Hot New Eye Drug Beovu Tied to Potential Vision Loss: Experts," (February 24, 2020), https://www.fiercepharma.com/pharma/retinal-society-flags-serious-side-effect-for-novartis-beovu (accessed December 30, 2021)	Herewith
	152.	FiercePharma, "The Top 20 Drugs by Worldwide sales in 2020," (May 3, 2021), https://www.fiercepharma.com/special-report/top-20-drugs-by-2020-sales (accessed September 26, 2022)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	153.	FocusVision, "Survey Sample Size: How Much Do I Need?" (April,11, 2019), https://www.focusvision.com/blog/survey-sample-size-how-much-do-i-need/ (accessed January 25, 2022)	Herewith
	154.	Fraser *et al.*, "The Role of Vascular Endothelial Growth Factor and Estradiol in the Regulation of Endometrial Angiogenesis and Cell Proliferation in the Marmoset," *Endocrinology*, 149(9), pp. 4413-4420 (May 2008) (electronic publication)	Herewith
	155.	Gagnon *et al.*, "The Cost of Pushing Pills: A New Estimate of Pharmaceutical Promotion Expenditures in the United States," PLoS Medicine, 5(1), pp. 29-33 (January 2008)	Herewith
	156.	Gallemore *et al.*, "When Anti-VEGF Treatment Fails: Retina Specialists Are Charting New Territory and Learning How to Spot and React to Failed Anti-VEGF Therapy," *Rev. Ophthalmology*, (March 2008)	Herewith
	157.	Genentech, Inc., "FDA Green-Lights Genentech's Lucentis for Macular Edema following Retinal Vein Occlusion," Press Release, (June 23, 2010), https://www.genengnews.com/news/fda-green-lights-genentechs-lucentis-for-macular-edema-following-retinal-vein-occlusion/ (accessed January 12, 2022)	Herewith
	158.	Genentech, Inc., "Genentech, Inc. Submits Biologics License Application For FDA Review Of Lucentis™ In Wet Age-Related Macular Degeneration," Press Release, (Dec. 30, 2005), https://www.biospace.com/article/releases/genentech-inc-submitsbiologics-license-application-for-fda-review-of-lucentis-tm-in-wetage- related-macular-degeneration-/ (accessed February 3, 2022)	Herewith
	159.	Gomez-Manzano *et al.*, "VEGF Trap Induces Antiglioma Effect at Different Stages of Disease," *NEURO-ONCOLOGY*, 10, pp. 940-945 (December 2008), cited in Deposition of Dr. Alexander M. Klibanov, Ph.D., on March 24, 2022	Herewith
	160.	GOOD DAYS, https://www.mygooddays.org/ (accessed May 18, 2022)	Herewith
	161.	Guha *et al.*, "The Economics of Commercial Success in Pharmaceutical Patent Litigation," *Landslide* 1(5) (2009)	Herewith
	162.	Hachiya *et al.*, "Increase in respiratory cost at high growth temperature is attributed to high protein turnover cost in Petunia x hybrida petals," *Plant, Cell, and Environment*, 30(10), pp. 1269-1283 (October 2007)	Herewith
	163.	Hanhart *et al.*, Correspondence regarding "Fellow Eye Effect of Unilateral Intravitreal Anti-VEGF Injections in Eyes with Diabetic Macular Edema," *EYE*, 29, pp. 292-293 (November 2014) (online publication), cited in Deposition of Dr. Alexander M. Klibanov, Ph.D., on March 24, 2022	Herewith
	164.	Hayes, "SEC Filings: Forms You Need To Know," *INVESTOPEDIA*, https://www.investopedia.com/articles/fundamental-analysis/08/sec-forms.asp (accessed January 20, 2021)	Herewith
	165.	HCPCS Codes, "HCPCS Codes," https://hcpcs.codes/ (accessed January 6, 2022)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	166.	Healio, "Access to Retina Providers Shows No Geographic Bias in U.S.," (March 12, 2019), https://www.healio.com/news/ophthalmology/20190312/access-to-retina-providers-shows-no-geographic-bias-in-us (accessed December 6, 2021)	Herewith
	167.	Hecht, "Ophthalmic Preparations," *Remington: The Science and Practice of Pharmacy*, Volume II, 19th edition, Chapter 89, pp. 1563-1576 (1995) (Easton, PA)	Herewith
	168.	Heier & FOCUS Study Group, *Abstract: Intravitreal Ranibizumab (Lucentis™) with Verteporfin Photodynamic Therapy for Neovascular Age-Related Macular Degeneration: Year One Results*, Am. Soc'y Retina Specialists Ann. Meeting 94 (2005)	Herewith
	169.	Heier *et al.*, "Intravitreal Aflibercept (VEGF Trap-Eye) in Wet Age-related Macular Degeneration," *Ophthalmology*, 119, Appendices 2-8, pp. 1-34 (December 2012), submitted in IPR2022-01524 as Exhibit 1030	Herewith
	170.	Heier, "VEGF Trap-Eye for Exudative AMD," *Retinal Physician*, (April 2009)	Herewith
	171.	Heimann, "Intravitreal Injections: Techniques and Sequelae," in *Medical Retina*, Holz & Spaide, eds., (2007) (New York, NY)	Herewith
	172.	Helzner, "Lucentis After 1 Year: Doctors praise this practice-transforming therapy — but find drawbacks," *Retinal Physician* (July 1, 2007), https://www.retinalphysician.com/issues/2007/july-aug/lucentis-after-1-year (accessed September 26, 2022)	Herewith
	173.	Highlights of Prescribing Information for Eylea (Revised: June 2021), cited in Deposition of Dr. Richard Manning, Ph.D., on May 4, 2022, submitted in IPR2021-00881 as Exhibit 1152	Herewith
	174.	Hirokawa *et al.*, "Tau Proteins: The Molecular Structure and Mode of Binding on Microtubules," *J. Cell Biol.*, 107(4), pp. 1449-1459 (October 1988)	Herewith
	175.	Hopkins Medicine, "Photodynamic Therapy for Age-Related Macular Degeneration," https://www.hopkinsmedicine.org/health/treatment-tests-and-therapies/photodynamic-therapy-for-agerelated-macular-degeneration (accessed December 1, 2021)	Herewith
	176.	Iacono *et al.*, "Antivascular Endothelial Growth Factor in Diabetic Retinopathy," *Dev. Ophthalmol.*, 46, pp. 39-53 (2010)	Herewith
	177.	IPR2021-00880, Corrected Patent Owner Response (February 11, 2022)	Herewith
	178.	IPR2021-00880, Patent Owner Sur-Reply (July 6, 2022)	Herewith
	179.	IPR2021-00880, Petitioner Reply (May 27, 2022) - [[REDACTED]]	Herewith
	180.	IPR2021-00881, Corrected Patent Owner Response (February 11, 2022) - [[REDACTED]]	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	181.	IPR2021-00881, Patent Owner Sur-Reply (July 6, 2022)	Herewith
	182.	IPR2021-00881, Petitioner Reply (May 27, 2022) - [[REDACTED]]	Herewith
	183.	IPR2022-01225, Paper 1, Petition for IPR (July 1, 2022)	Herewith
	184.	IPR2022-01226, Paper 1, Petition for IPR (July 1, 2022)	Herewith
	185.	IPR2022-01524, Paper 1, Petition for IPR (September 9, 2022)	Herewith
	186.	IQVIA, "Available IQVIA Data," https://www.iqvia.com/insights/the-iqvia-institute/available-iqvia-data (accessed January 18, 2022)	Herewith
	187.	IQVIA, Form 10-K, 2020	Herewith
	188.	Jaffe *et al.*, "Differential Response to Anti-VEGF Regimens in Age-Related Macular Degeneration Patients with Early Persistent Retinal Fluid," *Ophthalmology*, 123(9), pp. 1856-1864 (September 2016)	Herewith
	189.	Jager *et al.*, "Risks of Intravitreous Injection: A Comprehensive Review," *RETINA*, 24(5), pp. 676-698 (October 2004) (Philadelphia, PA)	Herewith
	190.	Johnson & Johnson Services, Inc., "Johnson & Johnson Completes Acquisition of Momenta Pharmaceuticals, Inc.," Press Release, (October 1, 2020), https://www.jnj.com/johnson-johnson-completes-acquisition-of-momenta-pharmaceuticals-inc (accessed August 2, 2021)	Herewith
	191.	Johnson & Johnson Services, Inc., "Johnson & Johnson to Acquire Momenta Pharmaceuticals, Inc., Expanding Janssen's Leadership in Novel Treatments for Autoimmune Diseases," Press Release, (August 19, 2020) https://www.jnj.com/johnson-johnson-to-acquire-momenta-pharmaceuticals-inc-expanding-janssens-leadership-in-novel-treatments-for-autoimmune-diseases (accessed August 2, 2021)	Herewith
	192.	Kaiser Family Foundation, "A Snapshot of Sources of Coverage Among Medicare Beneficiaries in 2018," available at: https://www.kff.org/medicare/issue-brief/a-snapshot-of-sources-of-coverage-among-medicare-beneficiaries-in-2018/ (accessed March 23, 2021)	Herewith
	193.	Kaiser Family Foundation, "Medicare Advantage in 2021: Enrollment Update and Key Trends," https://www.kff.org/medicare/issue-brief/medicare-advantage-in-2021-enrollment-update-and-key-trends/ (accessed June 21, 2021)	Herewith
	194.	Kanghong Pharmaceutical, "Announcement of Chengdu Kanghong Pharmaceutical Group Co., Ltd. on Stopping the Global Multi-center Clinical Trial of Conbercept Ophthalmic Injection," Press Release, http://epaper.zqrb.cn/html/2021-04/10/content_716426.htm?div=-1 (with English translation) (accessed September 26, 2022)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	195.	Kim *et al.*, "A Brief History of Anti-VEGF for the Treatment of Ocular Angiogenesis," *The American Journal of Pathology*, 181(2), pp. 376-379 (August 2012)	Herewith
	196.	Kim *et al.*, "Eyes that Do Not Meet the Eligibility Criteria of Clinical Trials on Age-Related Macular Degeneration: Proportions of the Real-World Patient Population and Reasons for Exclusion," *Journal of Ophthalmology*, 2021: Article ID 6635467, 8 pages (April 2021)	Herewith
	197.	Kleiger *et al.*, "The 1.7 Å Crystal Structure of BOI: A Study of How Two Dissimilar Amino Acid Sequences Can Adopt the Same Fold," *J. Mol. Biol.*, 299(4), pp. 1019-1034 (June 2000)	Herewith
	198.	Kuepper, "The Best Investment Information Sources: Using SEC Filings, Analyst Reports, and Company Websites," *The Balance*, https://www.thebalance.com/top-best-sources-of-investor-information-1979207 (accessed January 20, 2021)	Herewith
	199.	Kuhlmann *et al.*, "Lessons Learned from Biosimilar Epoetins and Insulins," *The British Journal of Diabetes & Vascular Disease*, 10(2), pp. 90-99 (April 2010)	Herewith
	200.	L36962: Medicare Part AB Local Coverage Determination (LCD) Comment Summary (May 2, 2014), cited in Deposition of Dr. David M. Brown, M.D., on April 26, 2022, submitted in IPR2021-00881 as Exhibit 1140	Herewith
	201.	Li *et al.*, "Safety and Efficacy of Conbercept in Neovascular Age-Related Macular Degeneration: Results from a 12-Month Randomized Phase 2 Study: AURORA Study," *Ophthalmology*, 121(9), pp. 1740-1747 (2014)	Herewith
	202.	Ling *et al.*, "Deregulating Direct-to-Consumer Marketing of Prescription Drugs: Effects on Prescription and Over-the-Counter Product Sales," *Journal of Law and Economics*, 45, pp. 691-723 (2002)	Herewith
	203.	Liu *et al.*, "A Novel Engineered VEGF Blocker with an Excellent Pharmacokinetic Profile and Robust Anti-Tumor Activity," *BMC CANCER*, 15(170), pp. 1-14 (March 2015) (online publication), cited in Deposition of Dr. Alexander M. Klibanov, Ph.D., on March 24, 2022	Herewith
	204.	Lucentis Label (revised April 2017), https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125156s114lbl.pdf (accessed September 26, 2022)	Herewith
	205.	Lucentis Label (revised August 2012), https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125156s0069s0076lbl.pdf (accessed September 26, 2022)	Herewith
	206.	Lucentis Label (revised June 2010), https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/125156s053lbl.pdf (accessed September 26, 2022)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	207.	Lucentis Label (revised March 2018), https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/125156s117lbl.pdf (accessed September 26, 2022)	Herewith
	208.	Macugen Approval Letter (December 17, 2004)	Herewith
	209.	Macugen Label (revised December 2004), https://www.accessdata.fda.gov/drugsatfda_docs/label/2004/021756lbl.pdf (accessed September 26, 2022)	Herewith
	210.	Macugen Label (submitted with NDA 21-756), submitted in IPR2021-00881 as Exhibit 2038	Herewith
	211.	Mankiw, *Principles of Microeconomics*, 5th ed., South-Western Cengage Learning (Mason, OH) (September 2009)	Herewith
	212.	Manning *et al.*, "Similar Products at Different Prices: Can Biopharmaceutical Companies Segment Markets?" *International Journal of the Economics of Business*, 22(2), pp. 231-243 (June 2015)	Herewith
	213.	Mathis, "Fine-Tuning Your Anti-VEGF Injection Protocols: The Second Article in Our Series Recapping Research and Analysis Presented at Our Annual Meeting," *Retinal Physician* (October 1, 2009), https://www.retinalphysician.com/issues/2009/october-2009/fine-tuning-your-anti-vegf-injection-protocols (accessed February 4, 2022)	Herewith
	214.	Mayo Clinic, "Wet Macular Degeneration Symptoms and Causes," https://www.mayoclinic.org/diseases-conditions/wet-macular-degeneration/symptoms-causes/syc-20351107 (accessed November 11, 2021)	Herewith
	215.	Mayo Clinic, "Wet Macular Degeneration," https://www.mayoclinic.org/diseases-conditions/wet-macular-degeneration/diagnosis-treatment/drc-20351113 (accessed November 11, 2021)	Herewith
	216.	Medicare Interactive, "Medicare Part B Covered Services," https://www.medicareinteractive.org/get-answers/medicare-covered-services/medicare-coverage-overview/summary-of-part-b-covered-services (accessed November 22, 2021)	Herewith
	217.	Medicare Interactive, "The Parts of Medicare (A, B, C, D)," https://www.medicareinteractive.org/get-answers/medicare-basics/medicare-coverage-overview/original-medicare (accessed November 30, 2021)	Herewith
	218.	Medicare.gov, "Macular Degeneration Tests & Treatment," https://www.medicare.gov/coverage/macular-degeneration-tests-treatment (accessed November 22, 2021)	Herewith
	219.	Medicare.gov, "Medicare Advantage Plans," https://www.medicare.gov/sign-up-change-plans/types-of-medicare-health-plans/medicare-advantage-plans (accessed December 31, 2021)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	220.	Medicare.gov, "When Does Medicare Coverage Start?" https://www.medicare.gov/basics/get-started-with-medicare/sign-up/when-does-medicare-coverage-start (accessed December 15, 2021)	Herewith
	221.	Medline Plus, "Laser Photocoagulation – Eye," https://medlineplus.gov/ency/article/007664.htm (accessed December 2, 2021)	Herewith
	222.	Miller & Zois, LLC, "Novartis Looking to Repurpose its Dangerous Beovu Drug," November 28, 2020, https://www.drugrecalllawyerblog.com/novartis-repurpose-beovu.html (accessed September 23, 2021)	Herewith
	223.	Miller, "Taking Advantage of the New Purple Book Patent Requirements for Biologics," (April 26, 2021), https://www.morganlewis.com/pubs/2021/04/taking-advantage-of-the-new-purple-book-patent-requirements-for-biologics (accessed September 26, 2022)	Herewith
	224.	Moroney *et al.*, "Aflibercept in Epithelial Ovarian Carcinoma," *Future Oncology*, 5(5), pp. 591-600 (June 2009)	Herewith
	225.	Mueller *et al.*, "Ocular Infection and Inflammation," EMERGENCY MED. CLINICS N. AM., 26(1), pp. 57-72 (February 2008) (Philadelphia, PA)	Herewith
	226.	Murphy *et al.*, "Protein Folding, Misfolding, Stability and Aggregation: An Overview," in *Misbehaving Proteins - Protein (Mis)Folding, Aggregation, and Stability*; Murphy *et al.*, eds., Springer, (2006) (New York, NY)	Herewith
	227.	Nieto *et al.*, "Ocular silicon distribution and clearance following intravitreal injection of porous silicon microparticles," *Exp. Eye Res.*, 116, pp. 161-168 (November 2013)	Herewith
	228.	Novartis Press Release, "Novartis Receives FDA Approval for Beovu, Offering Wet AMD Patients Vision Gains and Greater Fluid Reductions vs Aflibercept," (October 8, 2019), https://www.novartis.com/news/media-releases/novartis-receives-fda-approval-beovu-offering-wet-amd-patients-vision-gains-and-greater-fluid-reductions-vs-aflibercept (accessed September 26, 2022)	Herewith
	229.	Novartis Press Release, "US FDA Approves Updated Novartis Beovu Label, to Include Additional Safety Information," (June 11, 2020), https://www.novartis.com/news/media-releases/us-fda-approves-updated-novartis-beovu-label-include-additional-safety-information (accessed September 26, 2022)	Herewith
	230.	Novartis, Annual Report, 2020, submitted in IPR2021-00881 as Exhibit 2230	Herewith
	231.	Nucleic acid sequence alignment of SEQ ID NO:1 of the '338 and '069 patents with SEQ ID NO:15 of the '758 patent and SEQ ID NO:15 of the '959 patent, submitted in IPR2021-00881 as Exhibit 1124	Herewith
	232.	Nucleotide sequence alignment of SEQ ID NO:1 of the '338 patent with SEQ ID NO:15 of the '758 patent and SEQ ID NO:3 of Dix, submitted in IPR2022-00881 as Exhibit 1094	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	233.	Nucleotide sequence alignment of SEQ ID NO:1 of the '681 and '601 patents with SEQ ID NO:15 of the '758 patent and SEQ ID NO:1 of the '173 patent, submitted in IPR2022-01226 as Exhibit 1093	Herewith
	234.	Park *et al.*, "New Approach to Anti-VEGF Agents for Age-Related Macular Degeneration," *Journal of Ophthalmology*, 2012:Article ID 637316 (February 2012)	Herewith
	237.	Pflugfelder *et al.*, "Intravitreal Vancomycin: Retinal Toxicity, Clearance, and Interaction with Gentamicin," *Arch. Ophthalmol.*, 105(6), pp. 831-837 (June 1987)	Herewith
	238.	Pindyck *et al.*, *Microeconomics*, Upper Saddle River: Prentice Hall (2013)	Herewith
	239.	Piques *et al.*, "Ribosome and transcript copy numbers, polysome occupancy and enzyme dynamics in Arabidopsis," *Molecular Systems Biology*, 5(1), pp. 314 (January 2009)	Herewith
	240.	Prangé *et al.*, "Exploring Hydrophobic Sites in Proteins with Xenon or Krypton," *Proteins: Structure, Function, and Genetics*, 30(1), pp. 61-73 (January 1998)	Herewith
	241.	Publication of OIG Special Fraud Alerts, 59 Fed. Reg. 242, (December 19, 1994), https://oig.hhs.gov/documents/physicians-resources/980/121994.pdf (accessed September 26, 2022)	Herewith
	242.	Quiram *et al.*, "Exudative Age-Related Macular Degeneration: Current Therapies and Potential Treatments," *CLINICAL MEDICINE: THERAPEUTICS*, 1, pp. 1003-1011 (2009) (online publication)	Herewith
	243.	Ramazi *et al.*, "Post-translational modifications in proteins: resources, tools and prediction methods," *Database*, 2021(1):baab012 (April 2021)	Herewith
	244.	Regeneron Form 10-K for the year ended December 31, 2005, submitted in IPR2021-00881 as Exhibit 1147	Herewith
	245.	Regeneron Form 10-K for the year ended December 31, 2011, submitted in IPR2021-00881 as Exhibit 1149	Herewith
	246.	Regeneron Pharmaceuticals, Inc., "About," https://www.regeneron.com/about (accessed November 3, 2021)	Herewith
	247.	Regeneron Pharmaceuticals, Inc., "Eylea (aflibercept) Injection: Components of Reimbursement," 2015 - [[REDACTED]]	Herewith
	248.	Regeneron Pharmaceuticals, Inc., "Eylea Injection Receives FDA Approval for Macular Edema Following Retinal Vein Occlusion (RVO)," Press Release, (October 6, 2014) https://investor.regeneron.com/news-releases/news-release-details/eylear-aflibercept-injection-receives-fda-approval-macular-edema (accessed September 26, 2022)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	249.	Regeneron Pharmaceuticals, Inc., "Eylea Injection Receives FDA Approval for the Treatment of Diabetic Macular Edema (DME)," Press Release, (July 29, 2014) https://investor.regeneron.com/news-releases/news-release-details/eylear-aflibercept-injection-receives-fda-approval-treatment (accessed September 26, 2022)	Herewith
	250.	Regeneron Pharmaceuticals, Inc., "EYLEA," https://eylea.us/ (accessed May 18, 2022)	Herewith
	251.	Regeneron Pharmaceuticals, Inc., "FDA Approves Eylea Injection for Diabetic Retinopathy," (May 13, 2019) https://investor.regeneron.com/news-releases/news-release-details/fda-approves-eylear-aflibercept-injection-diabetic-retinopathy (accessed September 26, 2022)	Herewith
	252.	Regeneron Pharmaceuticals, Inc., "For the Treatment of Wet Age-Related Macular Degeneration," 2012	Herewith
	253.	Regeneron Pharmaceuticals, Inc., "History," https://www.regeneron.com/about/history (accessed December 15, 2021)	Herewith
	254.	Regeneron Pharmaceuticals, Inc., "Regeneron Announces FDA Approval of Eylea (Aflibercept) Injection for Macular Edema Following Central Retinal Vein Occlusion," Press Release, (September 21, 2012) https://investor.regeneron.com/news-releases/news-release-details/regeneron-announces-fda-approval-eylear-aflibercept-injection (accessed September 26, 2022)	Herewith
	255.	Regeneron Pharmaceuticals, Inc., "Regeneron Reports Fourth Quarter and Full Year 2012 Financial and Operating Results," Press Release (February 14, 2013), https://investor.regeneron.com/news-releases/news-release-details/regeneron-reports-fourth-quarter-and-full-year-2012-financial (accessed August 2, 2021)	Herewith
	256.	Regeneron Pharmaceuticals, Inc., "Regeneron Reports Fourth Quarter and Full Year 2019 Financial and Operating Results," Press Release, (February 6, 2020), https://investor.regeneron.com/news-releases/news-release-details/regeneron-reports-fourth-quarter-and-full-year-2019-financial (accessed August 2, 2021)	Herewith
	257.	Regeneron Pharmaceuticals, Inc., "Regeneron's Yancopoulos Receives Columbia College's Alexander Hamilton Award," Press Release, (November 22, 2019) https://www.prnewswire.com/news-releases/regenerons-yancopoulos-receives-columbia-colleges-alexander-hamilton-award-300963506.html (accessed September 26, 2022), cited in Deposition of Dr. Diana V. Do, M.D., on April 21, 2022	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	258.	Regeneron Pharmaceuticals, Inc., "Representative Regeneron U.S. Product Related Patents, EYLEA (aflibercept) Injection," (January 2022), https://www.regeneron.com/downloads/us-patent-products.pdf (accessed September 26, 2022), cited in Deposition of Dr. Richard Manning, Ph.D., on May 4, 2022	Herewith
	259.	Regeneron Pharmaceuticals, Inc., "Research Areas," https://www.regeneron.com/science/research-areas (accessed November 3, 2021)	Herewith
	260.	Regeneron Pharmaceuticals, Inc., "U.S. Eylea Historical Brand P&L," May 2021, submitted in IPR2021-00881 as Exhibit 2200 - [[REDACTED]]	Herewith
	261.	Regeneron Pharmaceuticals, Inc., "US Eylea P&L LTD," December 2021, submitted in IPR2021-00881 as Exhibit 2170 - [[REDACTED]]	Herewith
	262.	Regeneron Pharmaceuticals, Inc., ATU Sales Share Data: All Indications, 2021, submitted in IPR2021-00881 as Exhibit 2279	Herewith
	263.	Regeneron Pharmaceuticals, Inc., ATU Sales Share Data: BRVO, 2021, submitted in IPR2021-00881 as Exhibit 2283	Herewith
	264.	Regeneron Pharmaceuticals, Inc., ATU Sales Share Data: CRVO, 2021, submitted in IPR2021-00881 as Exhibit 2282	Herewith
	265.	Regeneron Pharmaceuticals, Inc., ATU Sales Share Data: DME, 2021, submitted in IPR2021-00881 as Exhibit 2281	Herewith
	266.	Regeneron Pharmaceuticals, Inc., ATU Sales Share Data: DR w/o DME, 2021, submitted in IPR2021-00881 as Exhibit 2284	Herewith
	267.	Regeneron Pharmaceuticals, Inc., ATU Sales Share Data: Wet AMD, 2021, submitted in IPR2021-00881 as Exhibit 2280	Herewith
	268.	Regeneron Pharmaceuticals, Inc., Earnings Call Transcript, April 26, 2012, submitted in IPR2021-00881 as Exhibit 2134	Herewith
	269.	Regeneron Pharmaceuticals, Inc., Earnings Call Transcript, February 13, 2012, submitted in IPR2021-00881 as Exhibit 2133	Herewith
	270.	Regeneron Pharmaceuticals, Inc., Earnings Call Transcript, July 25, 2012, submitted in IPR2021-00881 as Exhibit 2135	Herewith
	271.	Regeneron Pharmaceuticals, Inc., Eylea Gross & Net Sales P&L YTD, 2021, submitted in IPR2021-00881 as Exhibit 2285 - [[REDACTED]]	Herewith
	272.	Regeneron Pharmaceuticals, Inc., Eylea Marketing Material, 2013, submitted in IPR2021-00881 as Exhibit 2136	Herewith
	273.	Regeneron Pharmaceuticals, Inc., Eylea Marketing Material, November 2013, submitted in IPR2021-00881 as Exhibit 2137	Herewith
	274.	Regeneron Pharmaceuticals, Inc., Form 10-K, 2020, submitted in IPR2021-00881 as Exhibit 2254	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	275.	Retinal Physician, "Ongoing Treatment for Patients with Neovascular AMD," (October 1, 2007), https://www.retinalphysician.com/issues/2007/october-2007/ongoing-treatment-for-patients-with-neovascular-am (accessed September 26, 2022)	Herewith
	276.	Retinal Physician, "Retinal Physician Symposium Covers Broad Range of Topics," (September 1, 2006), https://www.retinalphysician.com/issues/2006/september-2006/retinal-physician-symposium-covers-broad-range-of (accessed February 4, 2022)	Herewith
	277.	Retinal Physician, "Revisiting an Early Treatment for Wet AMD: Is There a Role for Thermal Laser in the Era of Anti-VEGF Therapy?" Press Release, (September 1, 2011) https://www.retinalphysician.com/issues/2011/september-2011/revisiting-an-early-treatment-for-wet-amd (accessed September 26, 2022)	Herewith
	278.	Roche, "FDA Approves Lucentis for Treatment of Diabetic Macular Edema," Press Release, (August 13, 2012) https://www.roche.com/investors/updates/inv-update-2012-08-13.htm (accessed September 26, 2022)	Herewith
	279.	Roche, "FDA Approves Roche's Lucentis for Diabetic Retinopathy, the Leading Cause of Blindness Among Working Age Adults in the United States," Press Release, (April 18, 2017) https://www.roche.com/media/releases/med-cor-2017-04-18b.htm (accessed September 26, 2022)	Herewith
	280.	Roche, Finance Report, 2020, submitted in IPR2021-00881 as Exhibit 2256	Herewith
	281.	Rowe *et al.*, *Handbook of Pharmaceutical Excipients*, Cover to Preface (5th ed. 2006) (London, UK)	Herewith
	282.	Schneider, "Nits, Grits, and Soft Information in SEC Filings," *U. PA. L. REV.*, 121(2), pp. 254-305 (1972) (Philadelphia, PA)	Herewith
	283.	Schweitzer, *Pharmaceutical Economics and Policy: Second Edition*, Oxford University Press (2007) (New York, NY)	Herewith
	284.	ScienceDaily, "FDA Approves First Angiogenesis Inhibitor to Treat Colorectal Cancer," Press Release, (February 27, 2004) https://www.sciencedaily.com/releases/2004/02/040227071334.htm (accessed September 26, 2022)	Herewith
	285.	Shen *et al.*, "Clearance of Intravitreal Voriconazole," *Invest. Ophthalmology & Visual Sci.*, 45(5), pp. 2238-2241 (May 2007)	Herewith
	286.	Sivaprasad, "Sustained-Release Steroid Options For DME Therapy," *Retina Today*, pp. 34-36 (September 2021)	Herewith
	287.	Solá *et al.*, "Effects of Glycosylation on the Stability of Protein Pharmaceuticals," *Journal of Pharmaceutical Sciences*, 98(4), pp. 1223-1245 (April 2009)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	288.	Stefanini *et al.*, "Increase of Plasma VEGF after Intravenous Administration of Bevacizumab Is Predicted by a Pharmacokinetic Model," *CANCER RESEARCH*, 70(23), pp. 9886-9894 (December 2010), cited in Deposition of Dr. Alexander M. Klibanov, Ph.D., on March 24, 2022	Herewith
	289.	Thomas *et al.*, "Comparative Effectiveness of Aflibercept for the Treatment of Patients with Neovascular Age-related Macular Degeneration," *Clinical Ophthalmology*, 7, pp. 495-501 (March 2013)	Herewith
	290.	Thomson Reuters, "Thomson Reuters Links Discovery and Literature Citation Databases," Press Release (January 4, 2010)	Herewith
	291.	Transcript of Deposition of Doris Weber dated May 13, 2022, in IPR2021-00881	Herewith
	292.	Transcript of Deposition of Dr. Alexander M. Klibanov, Ph.D., dated March 24, 2022, in IPR2021-00880 and IPR2021-00881 - [[REDACTED]]	Herewith
	293.	Transcript of Deposition of Dr. David M. Brown, M.D., dated April 26, 2022, in IPR2021-00880 and IPR2021-00881	Herewith
	294.	Transcript of Deposition of Dr. Diana V. Do, M.D., dated April 21, 2022, in IPR2021-00881	Herewith
	295.	Transcript of Deposition of Dr. Lucian V. Del Priore, M.D., dated April 29, 2022, in IPR2021-00881 - [[REDACTED]]	Herewith
	296.	Transcript of Deposition of Dr. Richard Manning, Ph.D., dated May 4, 2022, in IPR2021-00881 - [[REDACTED]]	Herewith
	297.	Transcript of Deposition of Ivan Hofmann dated June 23, 2022, in IPR2021-00880 and IPR2021-00881 - [[REDACTED]]	Herewith
	298.	Transcript of Deposition of Mary Gerritsen, Ph.D., dated June 17, 2022, in IPR2021-00880 and IPR2021-00881	Herewith
	299.	Transcript of Deposition of Thomas Albini, M.D., dated June 22, 2022, in IPR2021-00880 and IPR2021-00881	Herewith
	300.	Transcript of the Teleconference before the United States Patent Trial and Appeal Board dated February 23, 2022, in IPR2021-00881	Herewith
	301.	Transcript of the Teleconference before the United States Patent Trial and Appeal Board dated May 19, 2022, in IPR2021-00880 and IPR2021-00881	Herewith
	302.	Transcript of the Teleconference before the United States Patent Trial and Appeal Board dated September 8, 2021, in IPR2021-00881	Herewith
	303.	U.S. Department of Health and Human Services (ASPE), "Medicare Part B Reimbursement of Prescription Drugs," 6/2014, available at: https://aspe.hhs.gov/sites/default/files/private/pdf/106966/ib_mprpd.pdf (accessed September 26, 2022)	Herewith
	304.	United Healthcare, "Ophthalmologic Policy: VEGF Inhibitors," effective January 1, 2022, submitted in IPR2021-00881 as Exhibit 1167	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	305.	USC-Brookings, "Medicare Payment for Physician-Administered (Part B) Drugs: The Interim Final Rule and a Better Way Forward," https://www.brookings.edu/blog/usc-brookings-schaeffer-on-health-policy/2021/02/10/medicare-payment-for-physician-administered-part-b-drugs/ (accessed September 26, 2022)	Herewith
	306.	Vanderkam, "George Yancopoulos: Doing Well by Trying to Do Good," *SCIENTIFIC AMERICAN*, https://www.scientificamerican.com/article/george-yancopoulos-westinghouse/ (accessed April 14, 2022), cited in Deposition of Dr. Diana V. Do, M.D., on April 21, 2022	Herewith
	307.	Verywell Health, "Macular Degeneration: Timeline of Vision Loss Progression," https://www.verywellhealth.com/macular-degeneration-timeline-5069947 (accessed March 21, 2021)	Herewith
	308.	Vestrum Health, "Pharmaceutical Companies," https://www.vestrumhealth.com/pharma.php (accessed January 3, 2022)	Herewith
	309.	Vestum Health, "Homepage," https://www.vestrumhealth.com/index.php (accessed January 3, 2022)	Herewith
	310.	Visudyne Label (revised April 2016), https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/021119s027lbl.pdf (accessed September 26, 2022)	Herewith
	311.	Volkin *et al.*, "Alterations in the Structure of Proteins that Cause Their Irreversible Inactivation," *Developments in Biological Standardization*, 74, pp. 73-81 (1992) (Basel, SI)	Herewith
	312.	Weidner *et al.*, "Observations Regarding the Average Sales Price Reimbursement Methodology," *Evidence-Based Oncology*, 27(4), pp. 156-160 (2021)	Herewith
	313.	Wells *et al.*, "Aflibercept, Bevacizumab, or Ranibizumab for Diabetic Macular Edema," *The New England Journal of Medicine*, 372(13), pp. 1193-1203 (2015)	Herewith
	314.	Wilhelmus, "The Red Eye, Infectious Conjunctivitis, Keratitis, Endophthalmitis, and Periocular Cellulitis," *INFECTIOUS DISEASE CLINICS N. AM.*, 2(1), pp. 99-116 (March 1988) (Philadelphia, PA)	Herewith
	315.	Wirbelauer, "Management of the Red Eye for the Primary Care Physician," *AM. J. MED.*, 119(4), pp. 302-306 (April 2006) (online publication)	Herewith
	316.	World Health Organization, "Blindness and Vision Impairment Fact Sheet," Press Release, (October 14, 2021) https://www.who.int/news-room/fact-sheets/detail/blindness-and-visual-impairment (accessed September 26, 2022)	Herewith
	317.	World Health Organization, "International Nonproprietary Names for Pharmaceutical Substances (INN)," *WHO Drug Information*, 20, pp. 118-119 (2006), cited in Deposition of Dr. Alexander M. Klibanov, Ph.D., on March 24, 2022	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	318.	Yahoo Finance, "Beovu Now Publicly Reimbursed in Ontario and New Brunswick for the Treatment of Neovascular Wet AMD," Press Release, (December 17, 2021) https://finance.yahoo.com/news/beovu-brolucizumab-injection-now-publicly-120000109.html (accessed December 30, 2021)	Herewith
	319.	Yang, "Comparison of Binding Characteristics and *in vitro* Activities of Three Inhibitors of Vascular Endothelial Growth Factor A," *Molecular Pharmaceutics*, 11(10), pp. 3421-3429 (October 2014), cited in Deposition of Dr. Alexander M. Klibanov, Ph.D., on March 24, 2022	Herewith
	320.	Yorston, "Anti-VEGF Drugs in the Prevention of Blindness," *Community Eye Health Journal*, 27(87), pp. 44-46 (2014)	Herewith
	321.	Zucchi, "EDGAR: Investors' One-Stop-Shop For Company Filings," *YAHOO!LIFE*, https://www.yahoo.com/lifestyle/tagged/health/edgar-investors-one-stop-shop-170000800.html (accessed January 20, 2021)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

Electronic Patent Application Fee Transmittal

Application Number:	17072417
Filing Date:	16-Oct-2020
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-008CIPCON6

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($)
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	1806	1	260	260
		Total in USD ($)		**260**

Electronic Acknowledgement Receipt

EFS ID:	46887120
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	25-OCT-2022
Filing Date:	16-OCT-2020
Time Stamp:	19:35:40
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$260
RAM confirmation Number	E20220OJ36211277
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	Transmittal Letter	REGN-008CIPCON6_2022-10-25_supp_IDS_trans.pdf	58284 40bdec380e906e81d349c09791ce5a5355f3aa27	no	5
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	REGN-008CIPCON6_2022-10_25_supp_IDS_1449.pdf	252307 8f96ec3386a677492c5f3f5bf49c1fc650fbd0a1	no	25
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Non Patent Literature	014_Ex_1066_EP-285.pdf	3939496 7499cd428f0cc868da081d032d92eedc5f2ffa16	no	28
Warnings:					
Information:					
4	Non Patent Literature	015-Ex2087-Abraham2010.pdf	1052234 e7cdf568d2210e4cb23c3777daac45386de0ad41	no	11
Warnings:					
Information:					
5	Non Patent Literature	016-Ex2084-AdamisChapter4.pdf	3999781 24d2d24ea12c1097bc6ea9abc3e6cd44493573f1	no	19
Warnings:					
Information:					
6	Non Patent Literature	017-Ex2141-AmAcadofOphthalmol-Anti-VEGFTreatments.pdf	1420930 46dbeb78d011a964bb4d6959f68803fb0726a9f2	no	3
Warnings:					

Information:					
7	Non Patent Literature	018_Ex_2142_Am_Acad_of_Ophthalmol-Bevacizumab.pdf	6114526 cbb0f6e277d98d1c41a376687c6b5afb985523a8	no	5
Warnings:					
Information:					
8	Non Patent Literature	019-Ex2143-AmAcadofOphthalmol-OphthalmologySubspecialists.pdf	839305 6cd08cdc103ddb8ae90a8703b9acb6baf33facc9	no	3
Warnings:					
Information:					
9	Non Patent Literature	021-Ex2145-AmAcadofOphthalmol-WhatIsAvastin.pdf	886057 03b6bd5fa5daf075bdcdf0e8ede3635c61fd27ae	no	4
Warnings:					
Information:					
10	Non Patent Literature	022-Ex2146-AmAcadofOphthalmol-WhatIsEylea.pdf	881994 85f598205a35832756d7136481b1c7e62b7c4215	no	4
Warnings:					
Information:					
11	Non Patent Literature	023-Ex2147-AmAcadofOphthalmol-WhatIsLucentis.pdf	885248 c60c64ceac7cac193f2a47c15e806136d614f35b	no	4
Warnings:					
Information:					
12	Non Patent Literature	024-Ex2148-AmSocofRetinaSpecialists-AboutUs.pdf	1002256 458d64234dcfd1cc1466d33eed4da4f106bb0fb9	no	3
Warnings:					
Information:					
13	Non Patent Literature	025-Ex2149-AmSocofRetinaSpecialists-Age-RelatedMacularDegeneration.pdf	1192024 6b3826352aa4cf4bc0a3dc467a13e2bb36e034f7	no	6
Warnings:					
Information:					

14	Non Patent Literature	026-Ex2150-AmSocofRetinaSpecialists-BranchRetinalVeinOcclusion.pdf	1137219 626d20ab22aa98e14471083 4aa52970c3503f97b	no	6
Warnings:					
Information:					
15	Non Patent Literature	027-Ex2151-AmSocofRetinaSpecialists-CentralRetinalVeinOcclusion.pdf	1861468 63ccc1687cf31ace8fa82941d886d399446b49f7	no	6
Warnings:					
Information:					
16	Non Patent Literature	028-Ex2152-AmSocofRetinaSpecialists-DiabeticRetinopathy.pdf	974104 02993dd4adfeaa3250d3ad5813ddcb45734f111b	no	7
Warnings:					
Information:					
17	Non Patent Literature	029-Ex2154-ASHA-CalculatingMedicareFeeScheduleRates.pdf	910724 a06100c09ba6267b87d06a747fdd475dc716a3a1	no	2
Warnings:					
Information:					
18	Non Patent Literature	031-Ex2153-Amgen-FusionProtein.pdf	723289 e9144ffffa6658f6bf0bc9dd31e9abfda421b302	no	2
Warnings:					
Information:					
19	Non Patent Literature	032-Ex1122-AAAlignment.pdf	282241 28747f2d2cafa6b28192f356f820823ba8e97dd6	no	1
Warnings:					
Information:					
20	Non Patent Literature	033_Ex_1093_338_amino_acid_sequences.pdf	327262 2fbd5fddcb93c5c9b3429617939c3d924268b9fd	no	1
Warnings:					
Information:					

21	Non Patent Literature	035_Ex_1087_AA_Alignment_vs_758_and_959_patents.pdf	364187 f7c95bb731f8e22052f740da5ce99c090d61de4e	no	1
Warnings:					
Information:					
22	Non Patent Literature	036_Ex_1092_AA_Alignment_vs_758_and_173_patents.pdf	286468 e4fb15bd7bee193a7c8fd7c9271306f580df393f	no	1
Warnings:					
Information:					
23	Non Patent Literature	040-Ex2157-BasePairBiotechnologies-WhatisanAptamer.pdf	1075672 846efa97a80745132ae6ea2136309515334 8f2dc	no	7
Warnings:					
Information:					
24	Non Patent Literature	041-Ex1155-Batta.pdf	678101 9adf195954f9cef801a24efeb4582e27b1eb b855	no	10
Warnings:					
Information:					
25	Non Patent Literature	043-Ex2158-BauschandLombWebsite-Visudyne.pdf	757256 d777896f8065dca05a706d7e6746acb3142 890ed	no	2
Warnings:					
Information:					
26	Non Patent Literature	044-Ex2160-BauschHealthCompanies10-K_2020.pdf	17692946 06e65cb3d3e8508ab29fecf4f2549c422f124 f01	no	168
Warnings:					
Information:					
27	Non Patent Literature	050-Ex2058-Bork2009.pdf	706814 a83eccdf8d076764fb628ae67b0708933aa0 b688	no	11
Warnings:					
Information:					

28	Non Patent Literature	051-Ex2164-BrightFocusFoundation-AMDFacts_Figures.pdf	900505 4e777ec943133385e73cabceb19f4165d170e4cd	no	5
Warnings:					
Information:					
29	Non Patent Literature	052-Ex2131-Brown2013.pdf	679184 0692b1b4cc518b89c6ea7ec4241e65150e64f57a	no	16
Warnings:					
Information:					
30	Non Patent Literature	053-Ex2085-Brown2009.pdf	1296284 612cb7910776c60a47f6642fac9faa037c39c210	no	14
Warnings:					
Information:					
31	Non Patent Literature	054-Ex2166-Calculatornet-SampleSizeCalculator.pdf	1247455 7d6a86493a56ad9fcb8398d32d7aac60c1d4c535	no	4
Warnings:					
Information:					
32	Non Patent Literature	055_Ex_1049_Capochiaro_2010.pdf	681881 2aceb62e9a322b9bc69e57a03d3b5d9af42c7d5d	no	13
Warnings:					
Information:					
33	Non Patent Literature	056-Ex2076-Cantu2010.pdf	1377043 df36a3846b170fb09c21cbf36866fc7ac8b2ae94	no	15
Warnings:					
Information:					
34	Non Patent Literature	058-Ex2091-LucentisLabel2006.pdf	986233 43178779df2f40a8d4c7fc0ac97d26dcc67116b1	no	8
Warnings:					
Information:					

35	Non Patent Literature	073-Ex2174-CMS-PhysicianFeeSchedule.pdf	843800 a08837d8ad992a8a6a756fc8e75489ac1010dee7	no	3
Warnings:					
Information:					
36	Non Patent Literature	074-Ex2172-CMS-2021ASPDrugPricingFiles.pdf	751768 554c493c78fe261d9669f56364a6229ac2a00a4e	no	2
Warnings:					
Information:					
37	Non Patent Literature	037-Ex_1138-DoDepEx4_338patentclaim1.pdf	324644 02e6b60208ec840072f4d79fcfb6bea764f39a16	no	1
Warnings:					
Information:					
38	Non Patent Literature	038-Ex1139-BrownDepEx10_ASRSClinicalUpdate.pdf	476770 a1aaeef3fa526da823f261ce30324ad426860bdf	no	2
Warnings:					
Information:					
39	Non Patent Literature	039-Ex2156-AvastinDrugLabel2004.pdf	352637 9e0db60e377038eecb6aa877f05bb0418c4a6ffb	no	27
Warnings:					
Information:					
40	Non Patent Literature	042-Ex2159-BauschandLombWebsite-VisudyneSafteyInformation.pdf	132154 5ca56ad441f48d6724aaaccb79bd1d0e357e4db0	no	2
Warnings:					
Information:					
41	Non Patent Literature	075-Ex2173-CMS-MedicarePartBDrugAverageSalesPrice.pdf	746952 f4f024a899503d70531953846d972c09ec15cb99	no	1
Warnings:					
Information:					

42	Non Patent Literature	076-Ex2077-Chen2016.pdf	737202 d6061128b5c7c605b834e8bfa1d8e85acdc670e5	no	12
Warnings:					
Information:					
43	Non Patent Literature	077_Ex_1059_Christensen.pdf	373458 19f14df6c670545df2dadb74a166ed6f44f30554	no	8
Warnings:					
Information:					
44	Non Patent Literature	078_Ex_1093_Brown_Dep_Ex_12_Clark.pdf	20838780 eead0527fef217fc4cd70926c12d570c2ef839a7	no	16
Warnings:					
Information:					
45	Non Patent Literature	049-Ex1172-BayerandRegenerontoCollaborateonVEGFTrap.pdf	103518 1e04d1635dde04f1b963b50f43d14b13ccefc66	no	9
Warnings:					
Information:					
46	Non Patent Literature	057-Ex1118-KlibanovDepEx9_CAS.pdf	976819 ab5cb3e8c711de9cd1eba031fa15ae19fbb92eec	no	3
Warnings:					
Information:					
47	Non Patent Literature	059_Ex_1050_Eylea_MR.pdf	24803946 c072eb13092476e605ac33a2ffee075dc7cdfb1d	no	206
Warnings:					
Information:					
48	Non Patent Literature	060-Ex2098-CDERStatisticalReview.pdf	6942530 3f318780b734410c6556d293bc3cc6232728fdb5	no	46
Warnings:					
Information:					

49	Non Patent Literature	061-Ex2167-CDC-VisionLoss-APublicHealthProblem.pdf	3073909 6d0b6799996a60693a86ef9124276a734ef41ac8	no	2
Warnings:					
Information:					
50	Non Patent Literature	062-Ex2168-CentersforMedicare_MedicaidServicesData-Practitioners.pdf	1249306 63cdd14f748b4b4c562aa80b0b6559302d0ce23b	no	4
Warnings:					
Information:					
51	Non Patent Literature	063-Ex2233-Oct12ASPPricingFilerevised060513.pdf	181431 c384a0c47d7d33c26f9a94764d6a1e876fce1486	no	10
Warnings:					
Information:					
52	Non Patent Literature	064-Ex2234-Oct13ASPPricingFileupdated060614.pdf	206899 36df5a0b1586672f660d241d4d8ffbe028b3ed5d	no	11
Warnings:					
Information:					
53	Non Patent Literature	065-Ex2240-Oct14ASPPricingFile-revised090115.pdf	173522 9d8b3c0915729633c273e88152e29d2e04f661ba	no	9
Warnings:					
Information:					
54	Non Patent Literature	066-Ex2235-Oct15ASPPricingFile-updated120915.pdf	174099 9cb5817fa2a42e0642180af8f61e75eaf281a5fb	no	9
Warnings:					
Information:					
55	Non Patent Literature	067-Ex2236-Oct16ASPPricingFileupdated082817.pdf	197903 cccee744894a3c633a63b7867ea1e6c018ed6f30	no	11
Warnings:					
Information:					

56	Non Patent Literature	068-Ex2237-Oct17ASPPricingFile083017.pdf	174392 053b332bc2a170813e6c6b16164677c6c1c9c3f5	no	11
Warnings:					
Information:					
57	Non Patent Literature	079-Ex2122-NCT00473330.pdf	199391 526640ea2167b243c66a755c441fbb760ce164fa	no	12
Warnings:					
Information:					
58	Non Patent Literature	069-Ex2238-Oct18ASPPricingFileupdated052919.pdf	176485 0bab6317d344be6a9941b1ed48045213ec252caf	no	11
Warnings:					
Information:					
59	Non Patent Literature	070-Ex2239-Oct2019ASPPricingFile052920.pdf	207009 e40f49f723dbac63f6fca75dad43f98ee3a69854	no	14
Warnings:					
Information:					
60	Non Patent Literature	071-Ex2241-October2020ASPPricingFileupdated090121.pdf	206025 461f49c78152c40b88e71f0b26e8fd9b520a3308	no	13
Warnings:					
Information:					
61	Fee Worksheet (SB06)	fee-info.pdf	38193 bb1c14dd2a998ec2a322637e7aaf19cfaef8dedc	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	122134320	

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	Attorney Docket No.	REGN-008CIPCON6
	Confirmation No.	7325
	First Named Inventor	George D. Yancopoulos
	Application Number	17/072,417
	Filing Date	October 16, 2020
	Group Art Unit	1647
Address to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Examiner Name	Jon McClelland Lockard
	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

The attention of the Examiner is invited to the documents listed on the attached Substitute 1449.

Copies of the U.S. patents and published applications listed on the attached Substitute 1449 are not submitted herewith, in accordance with the Strategic Plan Final Rule, 69 Fed. Reg. 56481-56547 (September 21, 2004), effective October 21, 2004.

Copies of the foreign publication and non-patent literature documents listed on the attached Substitute 1449 are submitted herewith.

It is respectfully requested that the information above be expressly considered during the prosecution of this application, and that the documents be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

No aspect of these submissions constitute admission of prior art status or a disclaimer of claim scope.

Statement under 37 C.F.R. §§1.56 and 1.2

Applicant hereby advises the Examiner of the status of a co-pending application(s) in compliance with the Applicant's duty to disclose under 37 C.F.R. §§1.56 and 1.2 (*see* also M.P.E.P. §2001.06(b)) as discussed in *McKesson Info. Soln. Inc., v. Bridge Medical Inc.*, 487 F.3d 897 (Fed. Cir. 2007).

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 13/940,370, filed July 12, 2013, which issued as U.S. Patent No. 9,254,338 on February 9, 2016.

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 14/972,560, filed December 17, 2015, which issued as U.S. Patent No. 9,669,069 on June 6, 2017.

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 15/471,506, filed March 28, 2017, which issued as U.S. Patent No. 10,130,681 on November 20, 2018.

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 16/055,847, filed August 6, 2018, which issued as U.S. Patent No. 10,857,205 on December 8, 2020.

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 16/159,282, filed October 12, 2018, which issued as U.S. Patent No. 10,828,345 on November 10, 2020.

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 16/397,267, filed April 29, 2019, which issued as U.S. Patent No. 10,888,601 on January 12, 2021.

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 17/352,892, filed June 21, 2021, which issued as U.S. Patent No. 11,253,572 on February 22, 2022.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 17/112,063, filed December 4, 2020. No actions have been mailed to date.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 17/112,404, filed December 4, 2020. No actions have been mailed to date.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 17/350,958, filed June 17, 2021. No actions have been mailed to date.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 17/740,744, filed May 10, 2022. A Non-Final Office Action was mailed on July 20, 2022.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention *Inter Partes* Review No. IPR2021-00880 of U.S. Patent No. 9,669,069, filed on May 5, 2021; and IPR2021-00881 of U.S. Patent No. 9,254,338, filed on May 5, 2021. Both of which are currently awaiting final decision from PTAB.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention *Inter Partes* Review No. IPR2022-01225 of U.S. Patent No. 10,130,681, filed on July 1, 2022; and IPR2022-01226 of U.S. Patent No. 10,888,601, filed on July 1, 2022.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention *Inter Partes* Review No. IPR2022-01524 of U.S. Patent No. 11,253,572, filed on September 9, 2022.

These documents and the corresponding file wrappers are available on PAIR or PTAB E2E, and thus are not provided with this communication. Please inform the undersigned if there is any difficulty in obtaining the documents from PAIR or PTAB E2E.

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-008CIPCON6.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: October 25, 2022

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
Reg. No. 28,807

BOZICEVIC, FIELD & FRANCIS LLP
201 Redwood Shores Parkway, Suite 200
Redwood City, CA 94065
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

Electronic Acknowledgement Receipt

EFS ID:	46890212
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	25-OCT-2022
Filing Date:	16-OCT-2020
Time Stamp:	19:40:10
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	Non Patent Literature	132-Ex_2183-Eye_Care_Surgery_Center-Macular_Degeneration.pdf	1249689 5710c647004e731604b1de0a325260130fdbc617	no	10

Warnings:

Information:					
2	Non Patent Literature	133_Ex_2184-EyeGuru-Intravitreal_Injection_Standard_Dosing_Table.pdf	781169 0e4e887783def4a0cd6d00d0c817097d8bf9ec3a	no	2
Warnings:					
Information:					
3	Non Patent Literature	138-Ex_2190-Eylea_Website_Dosing_Flexibility_for_Wet_AMD.pdf	1183499 3f4a2511e5192f879ca1a70c1384cf85e962cf2a	no	5
Warnings:					
Information:					
4	Non Patent Literature	139-Ex_2075-Eylea_Approval_Letter.pdf	1301999 4995947f092a8fbf196dbe9b712e814c193e7a0f	no	8
Warnings:					
Information:					
5	Non Patent Literature	140-Ex_1142-Fauser.pdf	234918 f3df520d02cb5658f86b85be816ac41fecbe590c	no	5
Warnings:					
Information:					
6	Non Patent Literature	141-Ex_1169-Eylea_Summary_Review.pdf	3492975 15191dd8b7c4cd6ce08e55bbfee67c84d1377816	no	29
Warnings:					
Information:					
7	Non Patent Literature	144-Ex_2108-FDA_Expedited_Programs.pdf	898809 3ea054cd4bed2936b8585d1c10ec2b779540d6d4	no	40
Warnings:					
Information:					
8	Non Patent Literature	145-Ex_2191-FDA_Website-Drug_Approval_Package_Macugen.pdf	864452 625b824f26dac6456868690249a6325c23a7da7d93	no	2
Warnings:					
Information:					

9	Non Patent Literature	146-Ex_2097-FDA_Non-Inferiority_Clinical_Trials.pdf	1187779 8c690862861ee9da4ea86274e85e3429f565ac1b	no	56
Warnings:					
Information:					
10	Non Patent Literature	148-Ex_2063-Fernandez-Ferreiro_2017.pdf	1128691 bd73c4000474f4e7fc4b8b2ab3defb3282834f06	no	9
Warnings:					
Information:					
11	Non Patent Literature	149-Ex_2055-Ferrara_2006.pdf	1766429 531de051af6136cbf042b89f9570989d6a38bfcd	no	12
Warnings:					
Information:					
12	Non Patent Literature	150-Ex_2192-FiercePharma-Beovu_Novartis.pdf	1007239 ac5555bafc032e194d3d3e7ddc0b5c8930493c64	no	4
Warnings:					
Information:					
13	Non Patent Literature	151-Ex_2193-FiercePharma-Novartis_Drug_Beovu_Tied_to_Potential_Vision_Loss.pdf	830837 4696ff6ba6188be82b5f2587d6425bfa550110bd	no	2
Warnings:					
Information:					
14	Non Patent Literature	153-Ex_2195-FocusVision-Survey_Sample_Size_How_Much_Do_I_Need.pdf	999840 474cbd6b803a66fa919aa5393cef730b4e0c1cae	no	4
Warnings:					
Information:					
15	Non Patent Literature	154_Ex_1036_Fraser.pdf	1058715 d573804fe393046769cfacbef4e7c99306df45ac	no	8
Warnings:					
Information:					

16	Non Patent Literature	155-Ex_2196-Gagnon_2008.pdf	1937735 85ef4d0ccc16cb902bc131309318d6a2e7596b2b	no	5
Warnings:					
Information:					
17	Non Patent Literature	156-Ex_2110-Gallemor_2008.pdf	896272 8c3075dd212618c601a1df4c53f7a63309710774	no	10
Warnings:					
Information:					
18	Non Patent Literature	157-Ex_2198-GEN-FDA_Green-Lights_Genentechs_Lucentis.pdf	818659 fc20160963d884a37f628d4403b8fc737e029e62	no	2
Warnings:					
Information:					
19	Non Patent Literature	134-Ex_2189-EyleaDrugLabelMar_2021.pdf	1127752 fd615b6d9e0c5af88a7763e8434424f132db7faf	no	32
Warnings:					
Information:					
20	Non Patent Literature	135-Ex_2019-May_2016_Eylea_Label.pdf	923888 972a4218a9c88c40b1c6b9721936fc8e4dd5bc9d	no	26
Warnings:					
Information:					
21	Non Patent Literature	136-Ex_2187-Eylea_Drug_Label_Oct_2014.pdf	678711 fd6b8655c7af62b723b0f10bd86ba84cb4fb7fbe	no	25
Warnings:					
Information:					
22	Non Patent Literature	137-Ex_2186-Eylea_Drug_Label_Sept_2012.pdf	577640 4fc9f74354a5b5fbc8984b186e940e7e839bbbed	no	19
Warnings:					
Information:					

23	Non Patent Literature	142-Ex_2099-FDA_22_Case_Studies.pdf	570169 2c992e52ad64103a81b787b7b318f97378cbf324	no	44
Warnings:					
Information:					
24	Non Patent Literature	143-Ex_1146-Drugs_FDA_Eylea.pdf	1161817 32fe93af7590e7e749bf357f986b2c6c27acfb29	no	3
Warnings:					
Information:					
25	Non Patent Literature	147-Ex_1166-Purple_Book_List.pdf	91409 ece25068ee4a053983d8cd82ea0b39248289045e	no	4
Warnings:					
Information:					
26	Non Patent Literature	152-Ex_2194-FiercePharma-The_top_20_drugs_by_worldwide_sales_in2020.pdf	665895 0acad227d1603c7539acd341d96a04b429a02f4b	no	2
Warnings:					
Information:					
27	Non Patent Literature	158-Ex_2120-GenentechPressReleaseDec_30_2005.pdf	110298 508361c7391d94eb5e665549c8727ce26f60c11e	no	11
Warnings:					
Information:					
28	Non Patent Literature	162_Ex_2016_Hachiya_2007.pdf	1183874 2e85799e17ac0b401610a3b9829e590d83750a8f	no	15
Warnings:					
Information:					
29	Non Patent Literature	164_Ex_1081-Hayes.pdf	928207 f3727b36832c368dfb17b0c0a71100fdd358162f	no	10
Warnings:					
Information:					

30	Non Patent Literature	165-Ex_2202-HCPCS_Codes-HCPCS_Codes.pdf	752416 05f8e8e151b8dfc2ae5bc6486ecf7fcb635cba23	no	1
Warnings:					
Information:					
31	Non Patent Literature	166-Ex_2203-Healio-Access_to_retina_providers_shows_no_geographic_bias_in_US.pdf	712562 bea80e1f0975627dd0afaa64b5054e3f0adc561d	no	1
Warnings:					
Information:					
32	Non Patent Literature	167-Ex_1025-Remington_Vol_2_opthalmic_solutions_1995_Hecht.pdf	2100807 ade40b5cbd2c3e0b98ffc8dabb0d923633a43048	no	18
Warnings:					
Information:					
33	Non Patent Literature	168-Ex_2100-Heier_2005.pdf	626580 252768a289a3d16b9edd4a4fac834739a20d8f54	no	1
Warnings:					
Information:					
34	Non Patent Literature	170-Ex_2080-Heier_Retinal_Physician_2009.pdf	751348 49fa2861b2714ffb606f555d8e37c9d68212adcd	no	7
Warnings:					
Information:					
35	Non Patent Literature	171-Ex1040-Heimann-2007.pdf	835498 c5b269d0c9968d0250cd793ee8cacbce0c08c8f4	no	23
Warnings:					
Information:					
36	Non Patent Literature	172-Ex_2106-Helzner2007.pdf	228728 0484ac193bbbe9bdaf14effcdbd20a8a2258078b	no	4
Warnings:					
Information:					

37	Non Patent Literature	174-Ex_2067-Hirokawa_1988.pdf	2841388 772d571cc240f10abf5763e6497bbdd95cbd3092	no	11
Warnings:					
Information:					
38	Non Patent Literature	175-Ex_2204-Hopkins_Medicine_Website-Photodynamic_Therapy_for_AMD.pdf	928472 604bf597621b8c053c02f3807af35053d35cb332	no	5
Warnings:					
Information:					
39	Non Patent Literature	176-Ex_2012-Iacono_2010.pdf	832397 32d77f20fa664afd39c6eaf30a965eb563e4b1f6	no	15
Warnings:					
Information:					
40	Non Patent Literature	159-Ex_1120-Klibanov_Dep_Ex_13_Gomez-Manzano.pdf	2654564 4826f2a9841ab2c4083a27cfa8ddd6861103dbb9	no	6
Warnings:					
Information:					
41	Non Patent Literature	163-Ex_1104-Klibanov_Dep_Ex_18_Hanhart.pdf	621315 d09a48908ced6660abe790b45d00ca7c5c938f42	no	2
Warnings:					
Information:					
42	Non Patent Literature	169-Ex_1030-Heier_2012-Appendix.pdf	311584 6b9268e559f9b47e535e55bd5c10f1af3c06585a	no	34
Warnings:					
Information:					
43	Non Patent Literature	173-Ex_1152-Manning_Dep_Ex_6_Eylea_Label_06_2021.pdf	3951557 8bee6b34e7d456b278c17ca2a09738f6700f2a53	no	10
Warnings:					
Information:					

44	Non Patent Literature	160-Ex_1170-mygoodays_org_CDF.pdf	2190789 ae1c652cb59037816d946de10b798356e9b46b0d	no	4
Warnings:					
Information:					
45	Non Patent Literature	161-Ex_2201-Guha2009.pdf	134197 cfca9a8f38689fa7326ce43010157e38e5ce3a3d	no	5
Warnings:					
Information:					
		Total Files Size (in bytes):	50133568		

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.

Electronic Acknowledgement Receipt

EFS ID:	46890736
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	25-OCT-2022
Filing Date:	16-OCT-2020
Time Stamp:	19:42:03
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	Non Patent Literature	174-Ex_2067-Hirokawa_1988.pdf	2841388 772d571cc240f10abf5763e6497bbdd95cbd3092	no	11

Warnings:

Information:					
2	Non Patent Literature	175-Ex_2204-Hopkins_Medicine_Website-Photodynamic_Therapy_for_AMD.pdf	928472 604bf597621b8c053c02f3807af35053d35cb332	no	5
Warnings:					
Information:					
3	Non Patent Literature	176-Ex_2012-Iacono_2010.pdf	832397 32d77f20fa664afd39c6eaf30a965eb563e4b1f6	no	15
Warnings:					
Information:					
4	Non Patent Literature	177-2022-02-11_38_CORRECTED_Patent_Owner_Response_IPR2021-00880_069_patent.pdf	567591 c1e633c29ad58f82f51ff21f3b7e6fbd706ca497	no	62
Warnings:					
Information:					
5	Non Patent Literature	178-2022-07-06_68_Patent_Owners_Sur-Reply-880.pdf	466526 931d5bc8a88e5301e2da58fda626ab5d8979b73d	no	57
Warnings:					
Information:					
6	Non Patent Literature	179-2022-05-27_57_Petitioner_Reply_REDACTED-880.pdf	423258 9883630efd0311a363a428fbd7d422544792d8a0	no	57
Warnings:					
Information:					
7	Non Patent Literature	180-2022-02-11_41_REDACTED_CORRECTED_Patent_Owner_Response_IPR2021-00881_338_patent.pdf	669797 29a567d45e42f459c6f0fd30aafb8e9a9d959046	no	95
Warnings:					
Information:					
8	Non Patent Literature	181-2022-07-06_73_Patent_Owners_Sur-Reply-881.pdf	551861 56625b1240e8890d222cf1e3d33101869dfa7dd0	no	68
Warnings:					
Information:					

9	Non Patent Literature	182-2022-05-27_62_Petitioner_Reply_REDACTED-881.pdf	723988 1d57d17cf6a134ea5e132d56a7568ecc9fbdd873	no	63
Warnings:					
Information:					
10	Non Patent Literature	183-2022-07-01_02_US_10130681_IPR_Petition-1225.pdf	817908 957c996f079319900325b3f1fb845e2a87f91525	no	91
Warnings:					
Information:					
11	Non Patent Literature	184-2022-07-01_02_US_10888601_IPR_Petition-1226.pdf	779279 1f29b0cb10e8d6838d378a94dc19fae252414dea	no	90
Warnings:					
Information:					
12	Non Patent Literature	185-2022-09-09_02_Apotex_Petition_IPR2022-01524.pdf	1408847 56a24c9d6fe244a49a4e4a73a3ab963f4c59fd48	no	86
Warnings:					
Information:					
13	Non Patent Literature	186-Ex_2207-IQVIA-AvailableIQVIAData.pdf	743412 56cff9ab402e6054211116f7d682d7c54af37c65	no	1
Warnings:					
Information:					
14	Non Patent Literature	187-Ex_2206-IQVIA_10-K_2020.pdf	2232939 29e8e91d80bc3c89681a28cdf6784c8eecff41e4	no	126
Warnings:					
Information:					
15	Non Patent Literature	188-Ex_2018-Jaffe_2016.pdf	1122011 793227ba5fa07d6454858ffc78967d0053754890	no	9
Warnings:					
Information:					

16	Non Patent Literature	193-Ex_2210-KFF-Medicare_Advantage_in_2021.pdf	2631717 608fbf24e434291a7cd97ad560acefb67e0be969	no	14
Warnings:					
Information:					
17	Non Patent Literature	190-Ex_2006-2020_10_01_JNJ_Press_Release.pdf	141410 fa5ffadc20f3bfcd60a212fd20d1a40d8eae624a	no	3
Warnings:					
Information:					
18	Non Patent Literature	192-Ex_2209-KFF-ASnapshotofSourcesofCoverageAmongMedicareBeneficiariesin2018.pdf	461363 61c144e9c9ea58535a799e64473ae51d197be629	no	9
Warnings:					
Information:					
19	Non Patent Literature	195-Ex_2056-Kim2012.pdf	816859 73337154e6db9e1ecba696356ab6190e16acc860	no	4
Warnings:					
Information:					
20	Non Patent Literature	198-Ex_1079-Kuepper.pdf	1328364 eb790a2cc6f4afdbf0ee2a8963c5eb4d2a8b27fa	no	5
Warnings:					
Information:					
21	Non Patent Literature	201-Ex_2027-Li_2014.pdf	7358199 0da8d3f480054852915239e634c186dfa81679de	no	8
Warnings:					
Information:					
22	Non Patent Literature	191-Ex_2005-2020_08_19_JNJ_Press_Release.pdf	159799 00ee697dc193e4dceee6eaf4dd30240e2a0c8388	no	4
Warnings:					
Information:					

23	Non Patent Literature	202-Ex_2211-Ling_2002.pdf	940324 12b88a13628393386c2d46169e5628aff981fd40	no	33
Warnings:					
Information:					
24	Non Patent Literature	204-Ex_2215-Lucentis_Drug_Label_Apr_2017.pdf	1059820 f00bc67e75936260f40b3a08c864ecc0ec14d4ce	no	27
Warnings:					
Information:					
25	Non Patent Literature	208-Ex_2117-Macugen_Approval_Letter.pdf	280192 fbb0c32da28c987d14a7d7ba62c1a8458acbab8d	no	4
Warnings:					
Information:					
26	Non Patent Literature	209-Ex_2217-Macugen_Drug_Label_Dec_2004.pdf	970756 2bd79e1a1fcced710480acb1469b44c01deb7591	no	8
Warnings:					
Information:					
27	Non Patent Literature	210-Ex_2038-2004_macugen_Label.pdf	1443683 a6c0fa8bc30cfb941087f4f1eef878463498475c	no	8
Warnings:					
Information:					
28	Non Patent Literature	211-Ex_2219-Mankiw_Chapter_1.pdf	6347658 b3d52e46f15eae4217e849fa88f3ae09d16aa1f6	no	45
Warnings:					
Information:					
29	Non Patent Literature	194-CombinedEx_2111and2112-KanghongPressRelease.pdf	232276 a207508ee71915093d679ea2536b1cb92789bf33	no	10
Warnings:					
Information:					

30	Non Patent Literature	196-Ex_2061-Kim_2021.pdf	967377 2674903928dfba36c2c896c158f84c3237c58eec	no	8
Warnings:					
Information:					
31	Non Patent Literature	197-Ex_2069-Kleiger_2000.pdf	908341 edad60a3b2967a81991b1b94ccb146fc40caef53	no	17
Warnings:					
Information:					
32	Non Patent Literature	199-Ex_2071-Kuhlmann_2010.pdf	521299 60b16a9986ad6a48cb90090fdead582e5029628e	no	10
Warnings:					
Information:					
33	Non Patent Literature	200-Ex_1140-Brown_Dep_Ex_11_Medicare_Comment_Summary.pdf	1454603 ec8df363232b296fd8fc30f7f312f8c487dbc7c7	no	4
Warnings:					
Information:					
34	Non Patent Literature	189-Ex_1057-Jager-2004.pdf	24129268 9a1a7e0eecd506f9df1887bd52d0a8b583d9aeb8	no	25
Warnings:					
Information:					
35	Non Patent Literature	203-Ex_1121-Klibanov_Dep_Ex_21_Liu.pdf	5434627 93e1f42aad09d079dfdde436e68f47e894e9543f	no	14
Warnings:					
Information:					
36	Non Patent Literature	205-Ex_2214-Lucentis_Drug_Label_Aug_2012.pdf	381881 965dd7564943a88630f96ea59bcb31c7474a457c	no	9
Warnings:					
Information:					

37	Non Patent Literature	206-Ex_2213-Lucentis_Drug_Label_June_2010.pdf	566810 2a88593f8151ead1df2220d2d45b065116eb5054	no	7
Warnings:					
Information:					
38	Non Patent Literature	207-Ex_2216-Lucentis_Drug_Label_Mar_2018.pdf	5901752 a4a650fb348bc95af70ec60c3564b78e656ec502	no	28
Warnings:					
Information:					
39	Non Patent Literature	212-Ex_2220-Manning_2015.pdf	2189121 da127e9c991d08d3f4dd53751a5d2dcd46309bda	no	14
Warnings:					
Information:					
40	Non Patent Literature	213-Ex_2104-Mathis_2009.pdf	479928 0aa1def7c6c3524d7c1805359b49c6739bf00184	no	10
Warnings:					
Information:					
41	Non Patent Literature	214-Ex_2222-Mayo_Clinic-Wet_macular_degeneration-Symptoms_and_Causes.pdf	1152724 1f0712616e3e3b305efc19abc0017d94e7185ae0	no	6
Warnings:					
Information:					
42	Non Patent Literature	215-Ex_2221-Mayo_Clinic-Wet_macular_degeneration-Diagnosis_and_treatment.pdf	1256463 0e9b66aea1da78dc1c34ce6599fb6ad1d9599045	no	9
Warnings:					
Information:					
43	Non Patent Literature	216-Ex_2223-Medicare_Interactive-Medicare_Part_B_Covered_Services.pdf	714260 28253d7980061d8a5c251ffdb84563850d1160af	no	1
Warnings:					
Information:					

44	Non Patent Literature	217-Ex_2224-Medicare_Interactive-The_parts_od_Medicare_A_B_C_D.pdf	739108 042c2325ca740ff499e365b2f75bf0d34e3bc3ff	no	1
Warnings:					
Information:					
45	Non Patent Literature	219-Ex_2226-Medicaregov-Medicare_Advantage_Plans.pdf	1009305 7bff213ffaae157f690db8f9b26e93da95a5f0ef	no	5
Warnings:					
Information:					
46	Non Patent Literature	220-Ex_2227-Medicaregov-When_does_Medicare_coverage_start.pdf	872193 abf5562ab095b6c81e379e3226ac1c1b968e5577	no	7
Warnings:					
Information:					
47	Non Patent Literature	221-Ex_2228-MedlinePlus_Medical_Encyclopedia-Laser_photocoagulation.pdf	801129 9854601eaa7409ce6079e14485b2877701b6ad58	no	4
Warnings:					
Information:					
48	Non Patent Literature	222-Ex_2180-Miller2020.pdf	806606 641047e2165bf3ed67feb2ee51c4631c84a7d2e5	no	3
Warnings:					
Information:					
49	Non Patent Literature	223-Ex_1175-MorganLewis-New_Purple_Book_Patent_Requirements.pdf	115301 2c82e0d8fd616c4f9872f955a49212c880970bf8	no	4
Warnings:					
Information:					
50	Non Patent Literature	224-Ex_2014_Moroney_2009.pdf	2615935 ec2e9856425e11a1069360d58d476357e5ba674d	no	10
Warnings:					
Information:					

51	Non Patent Literature	225-Ex_1060-Mueller.pdf	8574713 d3943062d9352cdb4728434e6bcc1c3cd520529a	no	18
Warnings:					
Information:					
52	Non Patent Literature	227-Ex_2062-Nieto_2013.pdf	993148 53a5d8b604f591484dd5c1075005b15a69fe5e1f	no	22
Warnings:					
Information:					
53	Non Patent Literature	228-Ex_2231-Novartis_Press_Release_Oct_8_2019.pdf	1398917 40360f551eb7cd98230c0627a390cb3c5e622afb	no	5
Warnings:					
Information:					
54	Non Patent Literature	230-Ex_2230-Novartis-Annual_Report_2020.pdf	5550495 4216f89679958ace109da58c1706b95e3dc2884b	no	300
Warnings:					
Information:					
55	Non Patent Literature	231-Ex_1124-NA_Alignment.pdf	617966 25515ed1474b4a379a7dcc9b20a061a9f89bf908	no	2
Warnings:					
Information:					
56	Non Patent Literature	232-Ex_1094-338_nucleotide_sequences.pdf	1347776 14d2056b99038905d42e553d44f8a480feb04159	no	2
Warnings:					
Information:					
57	Non Patent Literature	233-Ex_1093-NA_Alignment_vs_758_and_173_patents.pdf	988357 c58533db2fa8fbf24ef4acad1a0d20aba940624e	no	1
Warnings:					
Information:					

58	Non Patent Literature	234-Ex_2030-Park_2012.pdf	1323431 39bc5e92bd8922dfdbeaa355c67e4dded4b60be7	no	8
Warnings:					
Information:					
59	Non Patent Literature	237-Ex_2066-Pflugfelder_1987.pdf	3336760 468d77abc508a569905882ca4b867a7d9931a1a3	no	7
Warnings:					
Information:					
60	Non Patent Literature	218-Ex_2225-Medicare_gov-MacularDegenerationCoverage.pdf	556335 852f57a8b09ce04a5b03b549c663ba492f981f74	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	116988023	

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

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronic Acknowledgement Receipt

EFS ID:	46891432
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	25-OCT-2022
Filing Date:	16-OCT-2020
Time Stamp:	19:44:17
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	Non Patent Literature	229-Ex_2232-Novarti_Press_Release_June_11_2020.pdf	1458026 d5505b7349cc255fe395da02df61c1ed2138a324	no	5

Warnings:

Information:					
2	Non Patent Literature	238-Ex_2245-Pindyck_Chapter_3.pdf	6057248 72001b650151e5e5042e99b254d9dd789c68c64d	no	46
Warnings:					
Information:					
3	Non Patent Literature	239-Ex_2017-Piques_2009.pdf	1132273 43db0e45506d32d18d27c2e531a8e919b58409c7	no	17
Warnings:					
Information:					
4	Non Patent Literature	241-Ex_1174-oig_hhs_gov_Publication_of_OIG_Special_Fraud_Alerts.pdf	4020975 585b4de432e26ccdb4385f1965fc1cad8d3eb3be	no	11
Warnings:					
Information:					
5	Non Patent Literature	243-Ex_2057-Ramazi_2021.pdf	1241137 b3475a4b2d22c059aefe5db9b2a77e0cb9f15c41	no	20
Warnings:					
Information:					
6	Non Patent Literature	244-Ex_1147-Regeneron_2005_10-K.pdf	784522 ff0023547dde072a6f83ae428a2e703541df9450	no	102
Warnings:					
Information:					
7	Non Patent Literature	245-Ex_1149-Regeneron_2011_10-K.pdf	1191250 9e25a13b1de61add1ddadaa9544b1b8da3aaa02b	no	122
Warnings:					
Information:					
8	Non Patent Literature	246-Ex_2246-Regeneron-About_Us.pdf	1684545 7551f61c8bfc8a6aa40e8e2a28569fc16948974f	no	4
Warnings:					
Information:					

9	Non Patent Literature	247-Ex_2218-REDACTED_Eylea_Components_of_Reimbursement.pdf	8835824 e87d8e0f922018415fe5ec34ac01a14643cf6933	no	24
Warnings:					
Information:					
10	Non Patent Literature	248-Ex_2248-Regeneron_Press_Release__Oct_6_2014.pdf	830707 64ca41de5cd266959010b291f2ab177fb2d1835a	no	3
Warnings:					
Information:					
11	Non Patent Literature	249-Ex_2247-Regeneron_Press_Release_July_29_2014.pdf	879792 4968bb4930ab3b73b69e2baade5964867ea08756	no	4
Warnings:					
Information:					
12	Non Patent Literature	251-Ex_2249-Regeneron_Press_Release_May_13_2019.pdf	917050 d11cc1bbade3bc10eea2e6d6a91437b68c9d862e	no	4
Warnings:					
Information:					
13	Non Patent Literature	252-Ex_2139-Regeneron_For_the_Treatment_of_Wet_AMD_2012.pdf	8497918 60a9f002e80bf118a85064dbf8a9c0c123e8ed87	no	61
Warnings:					
Information:					
14	Non Patent Literature	253-Ex_2251-Regeneron-History.pdf	2372622 03254b99de8e8200815aecc361de3a909a1c6ae3	no	8
Warnings:					
Information:					
15	Non Patent Literature	254-Ex_2252-Regeneron_Press_Release_Sept_21_2012.pdf	851775 a23d874eaf95759d60292570480856ce7b12c943	no	3
Warnings:					
Information:					

16	Non Patent Literature	255-Ex_2039-2013_02_14_Regeneron_Press_Release.pdf	371652 b14de30b2ef7d008cd795899b24c341615c58921	no	6
Warnings:					
Information:					
17	Non Patent Literature	256-Ex_2040-Regeneron_Press_Release_Feb_6_2020.pdf	1142767 a589c811a330650b4e5538a8fd181539235cf4ba	no	15
Warnings:					
Information:					
18	Non Patent Literature	259_Ex_2253_Regeneron_Research_Areas.pdf	949849 3161c4057c9d3f27a7a5302294c5b7eb4efdc72c	no	2
Warnings:					
Information:					
19	Non Patent Literature	262-Ex_2279-Regeneron_ATU_Sales_Share_Data-All_Indications.pdf	689741 69a755506f1b8e06f9189cdc424ba7d586bcddd0	no	1
Warnings:					
Information:					
20	Non Patent Literature	263-Ex_2283-Regeneron_ATU_Sales_Share_Data-BRVO.pdf	690115 862344444a7d8a5d69a4cfe70452d089526cc0ae	no	1
Warnings:					
Information:					
21	Non Patent Literature	264-Ex_2282-Regeneron_ATU_Sales_Share_Data-CRVO.pdf	678293 8c0cde97450cd3b11e14817764232086986eeb2e	no	1
Warnings:					
Information:					
22	Non Patent Literature	265-Ex_2281-Regeneron_ATU_Sales_Share_Data-DME.pdf	678241 ce6e6ba0876db5658e54d74ec3a10e3a12b5d085	no	1
Warnings:					
Information:					

23	Non Patent Literature	266-Ex_2284-Regeneron_ATU_Sales_Share_Data-DR_without_DME.pdf	690578 6b761a8cb1d27b884b82bd7254703e295bbc39b8	no	1
Warnings:					
Information:					
24	Non Patent Literature	267-Ex_2280-Regeneron_ATU_Sales_Share_Data-Wet_AMD.pdf	677528 30e8cf474d47ab7538bb5b41274f311229472db2	no	1
Warnings:					
Information:					
25	Non Patent Literature	272-Ex_2136-Eylea_Marketing_Material_2013.pdf	1906317 4df5f235948491d9a4e599a4f0d7b1a0b093fb5e	no	2
Warnings:					
Information:					
26	Non Patent Literature	275-Ex_2103-Retinal_Physician-Ongoing_Treatment.pdf	708929 ab7cd474efa046e22bd1bfd9b7ace54d219f8a57	no	7
Warnings:					
Information:					
27	Non Patent Literature	277-Ex_2255-Retinal_Physician-Revisiting_an_Early_Treatment_for_Wet_AMD.pdf	2450141 c9203c7a9cbf38319bf7df2c11d79de3976f17fb	no	5
Warnings:					
Information:					
28	Non Patent Literature	278-Ex_2257-Roche_Press_Release_Aug_13_2012.pdf	1082471 24e2d855cff55225e49811c6e9cee8e255e34f5e	no	8
Warnings:					
Information:					
29	Non Patent Literature	280-Ex_2256-Roche_10-K_2020.pdf	7001327 ffabc6924d6df147854d58e6f5e0aba4e7816586	no	193
Warnings:					
Information:					

30	Non Patent Literature	282-Ex_1078-Schneider.pdf	4695956 b5702aa77ed9dd090babbe112f1f521ec5d6b5b1	no	52
Warnings:					
Information:					
31	Non Patent Literature	284-Ex_2261-ScienceDaily-FDA_Approves_First_Angiogenesis_Inhibitor.pdf	1311185 065ae09157c7071ebdaede981e71c1658bae0a3c	no	5
Warnings:					
Information:					
32	Non Patent Literature	285-Ex_2064-Shen_2007.pdf	604651 d22164b9d775a9f05a8a642c5a9993fed5d403b9	no	4
Warnings:					
Information:					
33	Non Patent Literature	286-Ex_2262-Sivaprasad_2021.pdf	3800739 28d3421ac26fcef7f994a84e8a9d657cfd74143a	no	3
Warnings:					
Information:					
34	Non Patent Literature	287-Ex_2074-Sola_2009.pdf	822342 40a3ee0c4eacff25c0a92d547050d0b56662460f	no	23
Warnings:					
Information:					
35	Non Patent Literature	289-Ex_2113-Thomas_2013.pdf	368710 bd1f4134949cda3e6c38cc33aec2e7a94e3cff74	no	7
Warnings:					
Information:					
36	Non Patent Literature	290-Ex_2043_Thomson_Reuters-881.pdf	1530052 d0ce8ee343d418cf41b2b7417d1a6c4ac73e7405	no	2
Warnings:					
Information:					

37	Non Patent Literature	291-Ex_1150-Weber_Dep_Tr.pdf	1246940 a3cdb9928c943a4c8e1845a6549e3a46fb501d21	no	159
Warnings:					
Information:					
38	Non Patent Literature	292_Ex_1108_Klibanov_Dep_Tr_REDACTED.pdf	1313479 b2bfba7ef63f77304b6c9e5fbc2807b3429ba614	no	104
Warnings:					
Information:					
39	Non Patent Literature	293_Ex_1110_Brown_Dep_Tr.pdf	1552978 e340f03808af78bbc20496043ff2f9e7fbc83018	no	214
Warnings:					
Information:					
40	Non Patent Literature	294_Ex_1109_Do_Tr.pdf	1673259 3868f28041d579a9d82fa28f7a21d01a4eb4f622	no	253
Warnings:					
Information:					
41	Non Patent Literature	295-Ex_1111-Del_Priore_Dep_Tr_REDACTED.pdf	1389069 5467e92e9f23e07d434c93c34868ef39cb7765d3	no	107
Warnings:					
Information:					
42	Non Patent Literature	296_Ex_1151-Manning_Dep_Tr_REDACTED.pdf	1622452 c9017e303e4b5ab020317b4ddaeaa850c8e2cb64	no	135
Warnings:					
Information:					
43	Non Patent Literature	300-Ex_1100-Transcript_23-February-2022-881.pdf	779681 4c8c6120f4a560097dd7c5aa14a64d9abca64ef0	no	27
Warnings:					
Information:					

44	Non Patent Literature	301-Ex_1116-Transcript_19-May-2022.pdf	923356 601d2316bfc8249b50232a161829c92318309745	no	59
Warnings:					
Information:					
45	Non Patent Literature	302-Ex_1097-Transcript_of_2021-09-08_Hearing.pdf	869750 bf99ab39b417ad2a1f8380b8b5803b49ec8e586b	no	29
Warnings:					
Information:					
46	Non Patent Literature	303-Ex_2155-ASPE_Issue_Brief-Medicare_Part_B_Reimbursement_of_Prescription_Drugs.pdf	1345125 b4c8ea4c3ab2a2ecb1e0e53424ed6fb506943c94	no	9
Warnings:					
Information:					
47	Non Patent Literature	304-Ex_1167-United_Healthcare_Ophthalmologic_Policy_VEGF.pdf	1183440 5984d79c1e686e585997acf8a03fccad8261ee99	no	40
Warnings:					
Information:					
48	Non Patent Literature	307-Ex_2264-Verywell_Health-Maclular_Degeneration_Timeline.pdf	1991261 26eafa5e1fa9c823a62450d7a72ece99f1d1021f	no	23
Warnings:					
Information:					
49	Non Patent Literature	309-Ex_2265-Vestrum_Health-Explore_what_we_can_do_for_you.pdf	739409 62457a31a4e6348b9b69fe0678c7b0f9f290c93c	no	2
Warnings:					
Information:					
50	Non Patent Literature	311-Ex_1126-Volkin_Klibanov_1992.pdf	4977672 c7281dff390d4fdd13af6abda57ec4e095c602ed	no	11
Warnings:					
Information:					

51	Non Patent Literature	313-Ex_2102-Wells_2015.pdf	1507378 3e9c4390bcdc38cc40ceab273f35456050f14c77	no	11
Warnings:					
Information:					
52	Non Patent Literature	314-Ex_1082-Wilhelmus-1988.pdf	9597776 d469bbd551a8f346d55637870aaf2244442 4874d	no	21
Warnings:					
Information:					
53	Non Patent Literature	257-Ex_1130-Do_Dep_Ex_2_Regeneron_22-Nov-2019.pdf	701429 7904db0aefaf2affca623ba58f8dddbbd5fd6c80	no	2
Warnings:					
Information:					
54	Non Patent Literature	258-Ex_1153-Manning_Dep_Ex_7_Eylea_Patent_List.pdf	189613 393cc3dc41e002aca30192606 4aba111786de7c7	no	1
Warnings:					
Information:					
55	Non Patent Literature	260-Ex_2200-REDACTED_Regeneron_US_Eylea_Historical_PL.pdf	514137 655443572a592d6ae0b8eda77d56971f183 28636	no	3
Warnings:					
Information:					
56	Non Patent Literature	261-Ex_2170-REDACTED_US_Eylea_P_L.pdf	109669 4f5af4cd282fe3afc8eab64049d0a282d632deeb	no	2
Warnings:					
Information:					
57	Non Patent Literature	268-Ex2134-Regeneron_Earnings_Call_Tr_Apr_26_2012.pdf	1312725 cc3794c35002fec5267beabf75ba2459a5f7badf	no	17
Warnings:					
Information:					

58	Non Patent Literature	269-Ex_2133-Regeneron_Earnings_Call_Tr_Feb_13_2012.pdf	1312293 1164684fadf532b8c9813acd96ff6956755d869e	no	16
Warnings:					
Information:					
59	Non Patent Literature	270-Ex_2135-Regeneron_Earnings_Call_Tr_July_25_2012.pdf	1394234 d43de003cdb5a8220023970908a113e9102d63e0	no	20
Warnings:					
Information:					
60	Non Patent Literature	271-Ex_2285-REDACTED_Eylea_Gross_Net_Sales_P_L.pdf	75711 69c723a7778c3afbaf4d1f930394fa1a7355b13b	no	1
Warnings:					
Information:					
		Total Files Size (in bytes):	111930084		

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.

Electronic Acknowledgement Receipt

EFS ID:	46891669
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	25-OCT-2022
Filing Date:	16-OCT-2020
Time Stamp:	19:45:50
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	Non Patent Literature	274-Ex_2254-Regeneron_Form_10-K_for_2020_2of2.pdf	22069351 afa3410028d46d6f435e4bfe8d1c9c0a0735da32	no	154

Warnings:

Information:					
2	Non Patent Literature	274-Ex_2254-Regeneron_Form_10-K_for_2020_1of2.pdf	25083024 0efbbb182fd8d07f3ea0a18609978085bc7cae7c	no	110
Warnings:					
Information:					
3	Non Patent Literature	273-Ex_2137-Eylea_Marketing_Material_Nov_2013_2of2.pdf	18768623 beff960e88d54bca5d0a77511e2b5f66fe41ae7e	no	16
Warnings:					
Information:					
4	Non Patent Literature	273-Ex_2137-Eylea_Marketing_Material_Nov_2013_1of2.pdf	15210475 5bf7a48b0d3c621ab6fbef7b8739a0d5eb5c988f	no	14
Warnings:					
Information:					
5	Non Patent Literature	276-Ex_2090-Retinal_Physician_Symposium.pdf	187017 32e06bff15c7a79700582eefc8fb97b6dc7948fa	no	8
Warnings:					
Information:					
6	Non Patent Literature	279-Ex_2258-Roche_Press_Release_Apr_18_2017.pdf	89719 7119fe6c2cbbb8be78d7cff0305d57e6004508cb	no	2
Warnings:					
Information:					
7	Non Patent Literature	281-Ex_1125-HPE_2006.pdf	889287 2fed89da09336d68304e0003f7fc08180660ef10	no	5
Warnings:					
Information:					
8	Non Patent Literature	283-Ex_2260-Schweitzer_Chapter_4.pdf	5548726 d60ab9edb2990395dcaaf4d6aed0d2c197a48877	no	20
Warnings:					
Information:					

9	Non Patent Literature	288-Ex_1105-Klibanov_Dep_Ex_19_Stefanini.pdf	12723930 e52e8b5159bd680289f8c0fcfb999c33bcffce16	no	9
Warnings:					
Information:					
10	Non Patent Literature	297_Ex_2289_PUBLIC_Ivan_Hofmann_Depo_Tr-881_2of2.pdf	21894632 a5205b19be380bfb62bc3fb7e8b86bf4e6335c58	no	82
Warnings:					
Information:					
11	Non Patent Literature	297_Ex_2289_PUBLIC_Ivan_Hofmann_Depo_Tr-881_1of2.pdf	19786696 6c4cfcaff0fb5c274a50d46354e5813c12fa3c95	no	77
Warnings:					
Information:					
12	Non Patent Literature	298-Ex_2288-Mary_Gerritsen_Depo_Tr-881.pdf	811250 c23b19530244194ccdd36b516f0e9d1667ac8e3a	no	207
Warnings:					
Information:					
13	Non Patent Literature	299-Ex_2287-Thomas_Albini_Depo_Tr-881.pdf	1132131 2f182fbbfba78fceefa1a98d2f9bc6925583ce57	no	311
Warnings:					
Information:					
14	Non Patent Literature	305-Ex_2165-Brookings-Medicare_Payment_for_Physician-Administered_Part_B_Drugs.pdf	160386 28657bb04ef095f0a758edba7b4ac115f7855292	no	2
Warnings:					
Information:					
15	Non Patent Literature	306-Ex_1131-Do_Dep_Ex_3_Vanderkam.pdf	8917036 87067aeb8db48730e4ee20023071a06b586e468a	no	4
Warnings:					
Information:					

16	Non Patent Literature	308-Ex_2266-Vestrum_Health-Pharmaceutical_companies.pdf	276624 6865f203563f782059abc1ef5d7e689e3f8ae662	no	3
Warnings:					
Information:					
17	Non Patent Literature	310-Ex_2267-VisudyneDrugLabelApr2016.pdf	261186 cb8dd159a6e90f12b21c858d18f1c507071943da	no	13
Warnings:					
Information:					
18	Non Patent Literature	312-Ex_2268-Weidner_2021.pdf	11849919 9c2bcec79d77b4b41f56e43a6fdb031cb9731e61	no	23
Warnings:					
Information:					
19	Non Patent Literature	314-Ex_1082-Wilhelmus-1988.pdf	9597776 d469bbd551a8f346d55637870aaf22444244874d	no	21
Warnings:					
Information:					
20	Non Patent Literature	315-Ex_1083-Wirbelauer-2006.pdf	842915 f59e4af9aab81a5e7770192514 78e821a65a1cf9	no	5
Warnings:					
Information:					
21	Non Patent Literature	316-Ex_2269-WHO-Blindness_and_vision_impairment.pdf	982054 4de9a65a225e626c0611b470386cd703d1f9ae3b	no	5
Warnings:					
Information:					
22	Non Patent Literature	318_Ex_2270_Yahoo_Finance_Beovu_Now_Publicly_Reimbursed.pdf	2428138 395e43dd80f5ca6017444fe8923949df230b68a5	no	8
Warnings:					
Information:					

23	Non Patent Literature	320_Ex_2271_Yorston_2014.pdf	1803653 a6b625620df318aa4e5d33f5144cff62e4bb1c0f	no	3
Warnings:					
Information:					
24	Non Patent Literature	321_Ex_1080_Zucchi.pdf	734357 f6b4bd33a11a55ad201ab60a6472a038a03ca876	no	4
Warnings:					
Information:					
25	Non Patent Literature	317_Ex_1107_Klibanov_Dep_Ex_22_WHO_Drug_Info.pdf	2369949 f19c7385cec0da5a31cd8ecccc4fe86dea813243	no	6
Warnings:					
Information:					
26	Non Patent Literature	319_Ex_1106_Klibanov_Dep_Ex_20_Yang.pdf	16903987 d78405b655594c5592eb3642573318e5738fbd6b	no	11
Warnings:					
Information:					
		Total Files Size (in bytes):	201322841		

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.

Electronic Acknowledgement Receipt

EFS ID:	46888190
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	25-OCT-2022
Filing Date:	16-OCT-2020
Time Stamp:	19:37:57
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	Non Patent Literature	045-Ex1168-BCBSFloridaVEGF.pdf	385719 7818fc7142a0b8a16b49476 4a22037312939a859	no	27

Warnings:

Information:					
2	Non Patent Literature	048-Ex2029Bhisitkul_Stewart2010.pdf	1037820 65fdb74b217252123de6c98cfd107ac57f81df00	no	11
Warnings:					
Information:					
3	Non Patent Literature	046-Ex2162-BeovuDrugLabelJune2020.pdf	473101 1de647bf2fc032ee32649e12d5fb2d37c4d6e90b	no	12
Warnings:					
Information:					
4	Non Patent Literature	047-Ex2161-BeovuDrugLabelOct2019.pdf	472292 3b213919a22b463402fd4e76239267c63ab18825	no	12
Warnings:					
Information:					
5	Non Patent Literature	072-Ex2242-October2021ASPPricingFileupdated011022.pdf	209564 7b307853c3671744fc887dc938dd16c39db47649	no	14
Warnings:					
Information:					
6	Non Patent Literature	020-Ex2144-AmAcadofOphthalmol-RetinalVasculitis.pdf	2414907 88e037cf3058492c5d6c20622a5dda017e78f6c4	no	9
Warnings:					
Information:					
7	Non Patent Literature	030-Ex1103-KlibanovDepEx3KlibanovAmgenDecl.pdf	20789709 0d0fa25f97b48f9f70a979709e439965b26880a7	no	75
Warnings:					
Information:					
8	Non Patent Literature	034-Ex1117-KlibanovDepEx6_73patentSEQIDNo2.pdf	476510 a041f02a8a26b1c1b928d673b6bf3193f5ed3801	no	2
Warnings:					
Information:					

9	Non Patent Literature	080-Ex2123-NCT00473382.pdf	199992 f0993746a026a3aa4aa22a361645c4f3f02a7924	no	12
Warnings:					
Information:					
10	Non Patent Literature	081-Ex2125-NCT00485836.pdf	182831 590b0067129a4f6601fde610f09e2853e1909d1e	no	9
Warnings:					
Information:					
11	Non Patent Literature	082-Ex2124-NCT00486018.pdf	181171 89a18a6fe6a28d6328ca6f53ee261cf00ee9d4ab	no	9
Warnings:					
Information:					
12	Non Patent Literature	083-Ex2078-NCT00519285.pdf	209194 4fc1cc518242099a3f61ce39d8fabe5913ead37a	no	13
Warnings:					
Information:					
13	Non Patent Literature	084-Ex_2079-NCT00644124.pdf	179848 e8d08edd6767ae4268cd5a2f883a2cd2ef154a98	no	8
Warnings:					
Information:					
14	Non Patent Literature	085-Ex_2053-NCT00794417.pdf	214470 1b6dd77ff687df4b3771120539ef2e20ff126231	no	9
Warnings:					
Information:					
15	Non Patent Literature	086-Ex_2054-NCT01148615.pdf	180561 2cdd4b8a085ff9afd2b745854e78d5835d917b4c	no	8
Warnings:					
Information:					

16	Non Patent Literature	087-Ex_2109-NCT01486771.pdf	183289 070118bf625ba0ff6702d6ede8e7490825b0f2dd	no	10
Warnings:					
Information:					
17	Non Patent Literature	088-Ex_2025-NCT01940900.pdf	194431 a683904c4b16188c7b460d3b5d40d3d3d16e9b95	no	7
Warnings:					
Information:					
18	Non Patent Literature	089-Ex_2024-NCT01944839.pdf	194985 62eadd9b93c26d8960793faf43db278c71454269	no	7
Warnings:					
Information:					
19	Non Patent Literature	090-Ex_2021-NCT02247479.pdf	214729 cb472cb2caea95853c791d72071497d785650b49	no	10
Warnings:					
Information:					
20	Non Patent Literature	091-Ex_2020-NCT02247531.pdf	214487 96fa7d5be5c78ae25d2c28d4ddd513cd6c955951	no	10
Warnings:					
Information:					
21	Non Patent Literature	092-Ex_2023-NCT03577899.pdf	204854 b60133c8dab7bcf51b0f893341473a917aa3fbde	no	9
Warnings:					
Information:					
22	Non Patent Literature	093-Ex_2022-NCT03630952.pdf	204920 b60ebea5025b65090baad0788a17840aad13905b	no	9
Warnings:					
Information:					

23	Non Patent Literature	094_Ex_1086_History_Clinical_Trials_gov.pdf	190836 e5d3fe11cdad97279ecc2fc9d8a9724e12d5fda6	no	9
Warnings:					
Information:					
24	Non Patent Literature	095_Ex_1069_Background_ClinicalTrials_gov.pdf	112443 5cfd13549df2c7d3e17e81cedf884e7da4286336	no	3
Warnings:					
Information:					
25	Non Patent Literature	096-Ex_2171-CloudResearch-Determining_Sample_Size.pdf	1348363 777a5dd04d1da88ca084b3c3f3337901f531a5b7	no	10
Warnings:					
Information:					
26	Non Patent Literature	097-Ex_2175-CMSMedicareCoverageDatabase-BillingandCodingAfilbercept.pdf	924714 3372ae2c14730069fdf7079cedcc67fba7a54632	no	7
Warnings:					
Information:					
27	Non Patent Literature	098-Ex_2065-Cobo1981.pdf	1090750 674d2de0614f1ae11bab8c447b5b26e2d0a90f9a	no	4
Warnings:					
Information:					
28	Non Patent Literature	101_Ex_1077_Corporate_Finance_Institute.pdf	374106 2d226911d4ca64bbe47383f4155e1cefcdc7d3de	no	3
Warnings:					
Information:					
29	Non Patent Literature	102_Ex_2105_Cousins_2010.pdf	977822 2b793a24893efa19adf285757dcbdb1f6a56913e	no	8
Warnings:					
Information:					

30	Non Patent Literature	104-Ex_1176-Dadgostar.pdf	290525 2f33aaa759ecadbe261cf1eead40015b61609dbe	no	8
Warnings:					
Information:					
31	Non Patent Literature	105-Ex_2286-SupplWeberDecl-881.pdf	817345 26aba71e3b06029602b82f9051ab245ab36190b0	no	7
Warnings:					
Information:					
32	Non Patent Literature	106-Ex_2070-Demarest2004.pdf	806693 30bc0c1f2657fbcf7fe0e1775e0414b24d4d6a25	no	8
Warnings:					
Information:					
33	Non Patent Literature	107-Ex_1143-Do.pdf	155469 7bb46dd5b15cf1ba67250372e996a7cb08eba8ec	no	5
Warnings:					
Information:					
34	Non Patent Literature	108-Ex_2177-Donahue2007.pdf	952421 c35c1363533d848f3f6e9fb62cb694e03b141066	no	9
Warnings:					
Information:					
35	Non Patent Literature	109-Ex_2178-Donohue2004.pdf	1512740 c7a3af619e640be6a56f07ff8d196c14851fd3d8	no	13
Warnings:					
Information:					
36	Non Patent Literature	110-Ex_2179-Dreyfuss_2015.pdf	1372586 315b8cd1832941503f0dcb4aa849b58fcd0a3658	no	3
Warnings:					
Information:					

37	Non Patent Literature	111-Ex_2181-Drugs_com-Eylea_FDA_Approval_History.pdf	692536 316e2b333498660e9c3becb6f31c2628e8c1bd56	no	1
Warnings:					
Information:					
38	Non Patent Literature	112-Ex_2115-FDA_Approves_Eylea_for_Wet_AMD.pdf	167745 01557e57438e7af7151ef6440a761f089884cd02	no	2
Warnings:					
Information:					
39	Non Patent Literature	114_Ex_2026-2017_08_14_BiopharmaDive_Article.pdf	134334 b7276e51d8f005aa9788d1ac55701f49351d8106	no	3
Warnings:					
Information:					
40	Non Patent Literature	115-Ex_2182-Elyasi_2021.pdf	1359129 6345c19dbaac6144b066019ede11a53d1875325e	no	3
Warnings:					
Information:					
41	Non Patent Literature	100-Ex_1154-2020-06-24Complaint.pdf	11052277 0a4018887cbef36a4b17369fa3d5198e3507cc5a	no	232
Warnings:					
Information:					
42	Non Patent Literature	103-Ex_2089-Cruz2006.pdf	100685 8da8976335228ed9cf7ca72c3a3d88b28e4541a4	no	3
Warnings:					
Information:					
43	Non Patent Literature	113-Ex_1112-Klibanov_Dep_Ex_14_Duncan.pdf	3268757 ecacfabfd4e5c361a5492afd532e15d3eac25444	no	8
Warnings:					
Information:					

44	Non Patent Literature	116_Ex_1067_EP-285-FH.pdf	10669150 4873902f205540ea9cc2d391ac87f559bab9b2cb	no	277
Warnings:					
Information:					
45	Non Patent Literature	117-Ex_2004-Momenta_SEC_Filing.pdf	1915845 fa9e2aba20a9b41b05c918c783745433188c1a34	no	66
Warnings:					
Information:					
46	Non Patent Literature	119_Ex_1002_Tanna_Declaration_APT_signed_090622.pdf	5332870 82bfc49fff4a6fc4fc292b9b82643ad039e25a16	no	107
Warnings:					
Information:					
47	Non Patent Literature	120-Ex_2050-Brown_Declaration_881.pdf	1877250 27c197bf2168ee289e5b3d8fe67b573b3d731c5d	no	92
Warnings:					
Information:					
48	Non Patent Literature	121-Ex_2049-REDACTED_Klibanov_Declaration_881.pdf	1359066 adc6c238aeca6c866d7777b3d14472bbe70bf360	no	55
Warnings:					
Information:					
49	Non Patent Literature	122-Ex_2051-Do_Declaration_881.pdf	1143035 ea745269725f9813ce3b70f732791b6a7bdb7f8c	no	67
Warnings:					
Information:					
50	Non Patent Literature	123-Ex_2048-REDACTED_Del_Priore_Declaration_881.pdf	1121845 7cb2062db54c9af3e7e6a7dba0f94b3ff7e8378f	no	56
Warnings:					
Information:					

51	Non Patent Literature	124_Ex_1002_Albini_Decl_681.pdf	3123989 ce381d6216401c3d6834187e6014f6ab2fc27e49	no	167
Warnings:					
Information:					
52	Non Patent Literature	125_Ex_1002_Albini_Decl_601.pdf	2872215 c912ff45c08c9fed3e634444f5207b86b04cbb28	no	165
Warnings:					
Information:					
53	Non Patent Literature	127-Ex_1137-Hofmann_Reply_Decl_REDACTED.pdf	530804 c90c0475a90df3be292a1418067711a5b1684140	no	76
Warnings:					
Information:					
54	Non Patent Literature	128_Ex_1003_Gerritsen_Decl_681.pdf	4655529 4cab8f0df64aadae55b425dd2df87645861bd2c4	no	59
Warnings:					
Information:					
55	Non Patent Literature	129_Ex_1003-Gerritsen_Decl_601.pdf	1995030 bf2b5b2e59ede2c9a5b9a297c5b8b2a1105f50b2	no	50
Warnings:					
Information:					
56	Non Patent Literature	130-Ex_1115-Gerritsen_Reply_Decl.pdf	957680 087fd83a5868fadceedd4f7a3acbe14aad518f6b	no	49
Warnings:					
Information:					
57	Non Patent Literature	131-Ex_2052-REDACTED_Manning_Declaration_with_Attachments_881.pdf	3133242 28622b2b33ce7df4aa5ab55fbff0a6925e1f98a7	no	289
Warnings:					
Information:					

58	Non Patent Literature	099-Ex1173-ComplaintHorizonvRegeneron 2022-04.pdf	15412174 ef924f6bfb5eeda467a8ebcf100c42dba99032cf	no	340
Warnings:					
Information:					
59	Non Patent Literature	118-Ex_2010_Biochemistry_2002_5_ed.pdf	11301568 9a523431b74efbea43171 8f9f943ac5478874b94	no	22
Warnings:					
Information:					
60	Non Patent Literature	126-Ex_1114_Albini_Reply_Decl.pdf	8732652 6e5681aadaf641aa4e358ec3caab3b16658bdf3c	no	109
Warnings:					
Information:					
			Total Files Size (in bytes):	132855604	

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 NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
17/072,417	10/16/2020	George D. YANCOPOULOS	REGN-008CIPCON6

CONFIRMATION NO. 7325

POA ACCEPTANCE LETTER

96387
Regeneron - Bozicevic, Field & Francis
201 REDWOOD SHORES PARKWAY
SUITE 200
REDWOOD CITY, CA 94065

OC000000136652923

Date Mailed: 10/31/2022

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 10/18/2022.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/snguyen/

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

U.S. PATENT DOCUMENTS					
		DOCUMENT NUMBER	DATE	NAME	REFERENCE PROVIDED*
	1	US 7,087,411 B2	08/08/2006	Daly *et al.*	not required per 69 Fed. Reg. 56481

FOREIGN PATENT DOCUMENTS						
		DOCUMENT NUMBER	DATE	COUNTRY	TRANSLATION	REFERENCE PROVIDED*

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	2	Berker *et al.*, "Surgical treatment of central retinal vein occlusion," *Acta Ophthalmol.*, 86:245-252 (2008)	Herewith
	3	Byeon *et al.*, "Short-Term Results of Intravitreal Bevacizumab for Macular Edema with Retinal Vein Obstruction and Diabetic Macular Edema, *J. OCULAR PHARMACOLOGY AND THERAPEUTICS*, 23(4):387-394 (November 2007)	Herewith
	4	ClinicalTrials.gov, "1997: Congress Passes Law (FDAMA) Requiring Trial Registration," (1997), https://clinicaltrials.gov/ct2/about-site/history, submitted in IPR2023-00099 as Exhibit 1085 (last updated May 2021)	Herewith
	5	Corrections to Kiire *et al.*, "Managing Retinal Vein Occlusion," *BMJ*, 344(e2110):1 (2012)	Herewith
	6	Expert Declaration of Dr. Jay M. Stewart in Support of Petition for *Inter Partes* Review of U.S. Patent No. 10,857,205 B2, dated October 27, 2022, in IPR2023-00099	Herewith
	7	Expert Declaration of Mary Gerritsen, Ph.D. in Support of Petition for *Inter Partes* Review of U.S. Patent No. 10,857,205 B2, dated October 27, 2022, in IPR2023-00099	Herewith
	8	Gewaily *et al.*, "Intravitreal steroids versus observation for macular edema secondary to central retinal vein occlusion," *Cochrane Database Syst. Rev.*, 1(CD007324):1-31 (2009)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	9	Golan *et al.*, "Current Treatment of Retinal Vein Occlusion," *Eur. Ophthalmic Rev.*, 5:62-68 (2011)	Herewith
	10	Keane *et al.*, "Retinal vein occlusion and macular edema – critical evaluation of the clinical value of ranibizumab," *Clinical Ophthalmology*, 5:771-781 (2011)	Herewith
	11	Kiire *et al.*, "Managing retinal vein occlusion," *BMJ*, 344(e499):1-16 (February 2012)	Herewith
	12	Kinge *et al.*, "Efficacy of Ranibizumab in Patients With Macular Edema Secondary to Central Retinal Vein Occlusion: Results From the Sham-Controlled ROCC Study," *American Journal of Ophthalmology*, 150(3):310-314 (2010)	Herewith
	13	Kreatsoulas, "Expanding Therapeutic Options for Retinal Vein Occlusion," *Retina Today*, pp. 20-21 (July/August 2009)	Herewith
	14	Petition for *Inter Partes* Review of U.S. Patent No. 10,857,205 B2, dated October 28, 2022, in IPR2023-00099	Herewith
	15	Pieramici, "Intravitreal Ranibizumab for Treatment of Macular Edema Secondary to Retinal Vein Occlusion," *Retina Today*, 44-46 (March 2009)	Herewith
	16	Regeneron Pharmaceuticals, Inc., "Bayer and Regeneron Extend Development Program for VEGF Trap-Eye to Include Central Retinal Vein Occlusion," Press Release, (Apr. 30, 2009), https://investor.regeneron.com/news-releases/news-release-details/bayer-and-regeneron-extend-development-program-vegf-trap-eye, submitted in IPR2023-00099 as Exhibit 1028 (last accessed November 4, 2022)	Herewith
	17	Regeneron Pharmaceuticals, Inc., "Regeneron and Bayer HealthCare Announce Encouraging 32-Week Follow Up Results from a Phase 2 Study of VEGF Trap-Eye in Age-Related Macular Degeneration," Press Release, (Apr. 28, 2008), http://newsroom.regeneron.com/releasedetail.cfm?releaseid=394066, submitted in IPR2023-00099 as Exhibit 1012 (last accessed November 11, 2022)	Herewith
	18	Regeneron Pharmaceuticals, Inc., "Regeneron Reports Third Quarter 2010 Financial Results and Business Highlights," Press Release (Oct. 28, 2010) https://investor.regeneron.com/news-releases/news-release-details/regeneron-reports-third-quarter-2010-financial-results-and, submitted in IPR2023-00099 as Exhibit 1058 (last accessed November 4, 2022)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	19	Regeneron Pharmaceuticals, Inc., Quarterly Report Pursuant to Section 13 or 15(d) of the Securities Exchange Act of 1934 (Form 10-Q) , submitted in IPR2023-00099 as Exhibit 1021 (Sept. 30, 2009)	Herewith
	20	Regeneron Pharmaceuticals, Inc., Quarterly Report Pursuant to Section 13 or 15(d) of the Securities Exchange Act of 1934 (Form 10-Q) , submitted in IPR2023-00099 as Exhibit 1022 (Sept. 30, 2010)	Herewith
	21	Shahid *et al.*, "The Management of Retinal Vein Occlusion: is Interventional Ophthalmology the Way Forward?," *Br. J. Ophthalmology*, 90:627-639 (2006)	Herewith
	22	Sophie *et al.*, "Aflibercept: a Potent Vascular Endothelial Growth Factor Antagonist for Neovascular Age-Related Macular Degeneration and Other Retinal Vascular Diseases," *Biol. Ther.*, 2(3):1-22 (2012)	Herewith
	23	Wu *et al.*, "Comparison Of Two Doses Of Intravitreal Bevacizumab (Avastin) For Treatment Of Macular Edema Secondary To Branch Retinal Vein Occlusion," *Retina*, 28:212-219 (2008)	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

SUBSTITUTE 1449 INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO.	APPLICATION NO.
	REGN-008CIPCON6	17/072,417
	APPLICANT	
	REGENERON PHARMACEUTICALS, INC.	
	FILING DATE	GROUP
	October 16, 2020	1647

NON-PATENT LITERATURE DOCUMENTS - FINAL WRITTEN DECISIONS			
		DOCUMENT (Including Author, Title, Date, Pertinent Pages, etc.)	REFERENCE PROVIDED*
	24	Final Written Decision Determining All Challenged Claims Unpatentable Denying Petitioner's Motion to Exclude Evidence Denying in part and Dismissing in Part Patent Owner's Motion to Exclude Evidence dated November 9, 2022, in IPR2021-00880 dated November 9, 2022, for US 9,669,069 B2	Herewith
	25	Final Written Decision Determining All Challenged Claims Unpatentable Denying in part and Dismissing in part Petitioners' Motion to Exclude Denying in part and Dismissing in part Denying Patent Owner's Motion to Exclude dated November 9, 2022, in IPR2021-00881 dated November 9, 2022, for US 9,254,338 B2	Herewith

EXAMINER	DATE CONSIDERED

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

*Copies of the listed references are either submitted herewith or were previously cited by or submitted to, the Office in a prior application. Pursuant to 37 C.F.R. § 1.97(d) and MPEP §609, the indicated reference may have been previously cited by or submitted to, the Office in a prior application, where the prior application is identified by its U.S. Application Number in this Information Disclosure Statement.

Electronic Patent Application Fee Transmittal

Application Number:	17072417
Filing Date:	16-Oct-2020
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-008CIPCON6

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($)
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	1806	1	260	260
		Total in USD ($)		**260**

Electronic Acknowledgement Receipt

EFS ID:	47020025
Application Number:	17072417
International Application Number:	
Confirmation Number:	7325
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/Kimberly Zuehlke
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON6
Receipt Date:	14-NOV-2022
Filing Date:	16-OCT-2020
Time Stamp:	16:57:13
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$260
RAM confirmation Number	E2022ADG58282155
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	Transmittal Letter	REGN-008CIPCON6_2022-11-14_supp_IDS_trans.pdf	58505 0638c193e00ed5aee1236f5e9aa32aea4ff966f3	no	5
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	REGN-008CIPCON6_2022-11-14_Substitute_1449.pdf	46337 e39266ace8e4ca8698b777b48457d6eef1d83386	no	4
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Non Patent Literature	02-Ex1043-Berker_and_Batman.pdf	4734236 11783e782209a023750fae36f5987812ee39d32e	no	8
Warnings:					
Information:					
4	Non Patent Literature	03-Ex1056-Byeon.pdf	363449 81a2da31517d66636cc6fde7114233ce18a7074f	no	8
Warnings:					
Information:					
5	Non Patent Literature	04-Ex1085-History-ClinicalTrials_gov.pdf	160469 1946d2d1ef4589fc26f9190bbd3321173fd20852	no	7
Warnings:					
Information:					
6	Non Patent Literature	05-Ex1089-Kiire-Corrections.pdf	246584 522a758b022742eaae21a42c269c4add20b139ed	no	1
Warnings:					

Information:					
7	Non Patent Literature	06-Ex1002-Stewart.pdf	12124003 f643a7a5ba3bd80fd68d5149d450d08ffee2b522	no	100
Warnings:					
Information:					
8	Non Patent Literature	07-Ex1003-Gerritsen.pdf	3163501 95672421490d036a36e756119bf8943ebae564ea	no	38
Warnings:					
Information:					
9	Non Patent Literature	08-Ex1042-Gewaily_and_Greenberg.pdf	403650 a4beffbf53dc43e4d0f15cf1479611d38af2188b	no	31
Warnings:					
Information:					
10	Non Patent Literature	09-Ex1039-Golan_OPTIMIZED.pdf	13610640 874c5642c76f7dc2d61222bd2e3dc6da66628eb3	no	7
Warnings:					
Information:					
11	Non Patent Literature	10-Ex1050-Keane_2011.pdf	480274 9f20c609972facdcef9bb0edea8eb7be87a510c4	no	11
Warnings:					
Information:					
12	Non Patent Literature	11-Ex1038-Kiire.pdf	12426923 7165d940b1fb0454d7b2e25dc1d5aad2ee4b6074	no	16
Warnings:					
Information:					
13	Non Patent Literature	12-Ex1053-Kinge.pdf	15842296 5ed0f4cadbc749e1295a2d3bc0a338d2b58bf5ee	no	5
Warnings:					
Information:					

14	Non Patent Literature	13-Ex1049-Kreatsoulas.pdf	5248067 2f6bc86f32184821bac452bfaed0d46e2acba7c4	no	2
Warnings:					
Information:					
15	Non Patent Literature	14-2022-10-28_02_Petition_for_IPR_of_205_Patent.pdf	783250 f05386eb86797075e7b60eb3361d668d32698b10	no	90
Warnings:					
Information:					
16	Non Patent Literature	15-Ex1054-Pieramici.pdf	9190945 5753b643b96a46064e89eae4f47d5ec0571f7d73	no	3
Warnings:					
Information:					
17	Non Patent Literature	16-Ex1028-Regeneron_30-April-2009.pdf	114457 81083c819ca3f164a4f508804227cd787fb9c5a4	no	3
Warnings:					
Information:					
18	Non Patent Literature	17-Ex1012-Regeneron_28-April-2008.pdf	596273 ad9debbc44bae77c2c6f16bf40be3805195e6d7e	no	3
Warnings:					
Information:					
19	Non Patent Literature	18-Ex1058-Regeneron_28-October-2010.pdf	157343 13c3012c7e30e00a0b1a99e16ea7d1d1b768daad	no	5
Warnings:					
Information:					
20	Non Patent Literature	19-Ex1021-2009_10-Q.pdf	535516 f5bbcce8028a0c0898e049d98a92ff653ee1c0a6	no	55
Warnings:					
Information:					

21	Non Patent Literature	20-Ex1022-2010_10-Q.pdf	530655 960710f67e94e8c7eb236d6cc1497b63faad760d	no	59
Warnings:					
Information:					
22	Non Patent Literature	21-Ex1032-Shahid.pdf	426529 86b4bb735963a20a51604fa91b4b73fc1cee5b36	no	13
Warnings:					
Information:					
23	Non Patent Literature	22-Ex1010-Sophie.pdf	421327 56d51203b27e61c4919246e583a14bccff8922ed	no	22
Warnings:					
Information:					
24	Non Patent Literature	23-Ex1055-Wu-min.pdf	3215212 f1dca829a3bf8bd9c926d64e1d70cee12daacf45	no	8
Warnings:					
Information:					
25	Non Patent Literature	24-2022-1-09_89_Final_Written_Decision_on_069_patent.pdf	526654 a515f72baac2611bc0a9c7a733ad7eb38dacf4ee	no	76
Warnings:					
Information:					
26	Non Patent Literature	25_2022-11-09_94_Final_Written_Decision_on_338_patent.pdf	493619 937365928071fb36cdad75ee3ba3c9ca1f79c655	no	64
Warnings:					
Information:					
27	Fee Worksheet (SB06)	fee-info.pdf	38193 7d9da85971eb111087891e922241546ac9d6792b	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	85938907	

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	Attorney Docket No.	REGN-008CIPCON6
	Confirmation No.	7325
	First Named Inventor	George D. Yancopoulos
	Application Number	17/072,417
	Filing Date	October 16, 2020
	Group Art Unit	1647
	Examiner Name	Jon McClelland Lockard
Address to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

The attention of the Examiner is invited to the documents listed on the attached Substitute 1449.

A copy of the U.S. patent listed on the attached Substitute 1449 is not submitted herewith, in accordance with the Strategic Plan Final Rule, 69 Fed. Reg. 56481-56547 (September 21, 2004), effective October 21, 2004.

Copies of the non-patent literature documents listed on the attached Substitute 1449 are submitted herewith.

It is respectfully requested that the information above be expressly considered during the prosecution of this application, and that the documents be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

No aspect of these submissions constitute admission of prior art status or a disclaimer of claim scope.

Statement under 37 C.F.R. §§1.56 and 1.2

Applicant hereby advises the Examiner of the status of a co-pending application(s) in compliance with the Applicant's duty to disclose under 37 C.F.R. §§1.56 and 1.2 (*see* also M.P.E.P. §2001.06(b)) as discussed in *McKesson Info. Soln. Inc., v. Bridge Medical Inc.*, 487 F.3d 897 (Fed. Cir. 2007).

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 13/940,370, filed July 12, 2013, which issued as U.S. Patent No. 9,254,338 on February 9, 2016.

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 14/972,560, filed December 17, 2015, which issued as U.S. Patent No. 9,669,069 on June 6, 2017.

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 15/471,506, filed March 28, 2017, which issued as U.S. Patent No. 10,130,681 on November 20, 2018.

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 16/055,847, filed August 6, 2018, which issued as U.S. Patent No. 10,857,205 on December 8, 2020.

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 16/159,282, filed October 12, 2018, which issued as U.S. Patent No. 10,828,345 on November 10, 2020.

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 16/397,267, filed April 29, 2019, which issued as U.S. Patent No. 10,888,601 on January 12, 2021.

With respect to this statement under *McKesson*, Applicants wishes to bring to the Examiner's attention U.S. Patent Application No. 17/352,892, filed June 21, 2021, which issued as U.S. Patent No. 11,253,572 on February 22, 2022.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 17/112,063, filed December 4, 2020. A Non-Final Office Action issued on October 11, 2022. A response thereto has not yet been filed.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 17/112,404, filed December 4, 2020. A Non-Final Office Action issued on October 27, 2022. A response thereto has not yet been filed.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 17/350,958, filed June 17, 2021.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention U.S. Patent Application No. 17/740,744, filed May 10, 2022. A Notice of Allowance issued on November 14, 2022. A response thereto has not yet been filed.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention *Inter Partes* Review Application No. IPR2021-00880 of U.S. Patent No. 9,669,069, filed on May 5, 2021. A Final Written Decision dated November 9, 2022, has been issued by PTAB.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention *Inter Partes* Review Application No. IPR2021-00881 of U.S. Patent No. 9,254,338, filed on May 5, 2021. A Final Written Decision dated November 9, 2022, has been issued by PTAB.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention *Inter Partes* Review Application No. IPR2022-01225 of U.S. Patent No. 10,130,681, filed on July 1, 2022; IPR2022-01226 of U.S. Patent No. 10,888,601, filed on July 1, 2022.

With respect to this statement under *McKesson*, Applicant wishes to bring to the Examiner's attention *Inter Partes* Review Application No. IPR2023-00099 of U.S. Patent No. 10,857,205, filed on October 28, 2022.

These documents and the corresponding file wrappers are available on PAIR or PTAB E2E, and thus are not provided with this communication. Please inform the undersigned if there is any difficulty in obtaining the documents from PAIR or PTAB E2E.

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-008CIPCON6.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 14 November 2022

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
Reg. No. 28,807

BOZICEVIC, FIELD & FRANCIS LLP
201 Redwood Shores Parkway, Suite 200
Redwood City, CA 94065
Telephone: (650) 327-3400
Facsimile: (650) 327-3231