Electronically filed		
PRELIMINARY AMENDMENT UNDER 37 C.F.R. §1.115	Attorney Docket No.	REGN-008CIPCON4
	Confirmation No.	8618
	First Named Inventor	George D. Yancopoulos
	Application Number	16/159,282
	Filing Date	October 12, 2018
	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:

This Preliminary Amendment is being submitted concurrently with a Request for Continued Examination (RCE). In view of the remarks put forth below, reconsideration and allowance are respectfully requested.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 4 of this paper.

AMENDMENTS TO THE CLAIMS

1. - 31. (**Canceled**)

32. (Previously Presented) 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 4 weeks after the immediately preceding dose; and

wherein each tertiary dose is administered 12 weeks after the immediately preceding dose;

wherein the VEGF antagonist is a receptor-based chimeric molecule comprising an immunoglobin-like (Ig) domain 2 of a first VEGF receptor which is Flt1 and Ig domain 3 of a second VEGF receptor which is Flk1, and a multimerizing component.

33. (Previously Presented) The method of claim 32, wherein the VEGF antagonist is aflibercept.

34. (Previously Presented) The method of claim 32, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

35. (Previously Presented) The method of claim 34, wherein the intraocular administration is intravitreal administration.

36. (Previously Presented) The method of claim 35, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

37. (Previously Presented) The method of claim 36, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

38. (Previously Presented) The method of claim 36, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

39. (Previously Presented) The method of claim 36, 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.

40. (Previously Presented) The method of claim 39 wherein the angiogenic eye disorder is age related macular degeneration.

41. (Previously Presented) The method of claim 39 wherein the angiogenic eye disorder is diabetic retinopathy.

42. (Previously Presented) The method of claim 39, wherein the angiogenic eye disorder is diabetic macular edema.

REMARKS

FORMAL MATTERS

Claims 32-42 are pending in this application

Claims 1-31 were previously cancelled.

No claims are amended.

No new matter is added.

ALLOWED CLAIMS

The claims that are pending here and shown above are identical to the claims that were allowed in the Notice of Allowance dated April 1, 2020.

This request for continued examination is filed for the purpose of citing additional publications in an IDS and thereby fully complying with Applicant's duty of disclosure.

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

Applicant hereby advises 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 Request for Continued Examination was filed on June 30, 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.

*This Statement is not an admission that any of the listed patents/applications are relevant to the instant claims.

CONCLUSION

Applicant submits that all the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-008CIPCON4.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 30 June 2020

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

INFORMATION DISCLOSURE STATEMENT	Attorney Docket No.	REGN-008CIPCON4
	Confirmation No.	8618
	First Named Inventor	George D. Yancopoulos
	Application Number	16/159,282
	Filing Date	October 12, 2018
	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:

Applicants submit herewith documents which may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 C.F.R. § 1.56. This submission is not intended to constitute an admission that any document referred to therein is "prior art" for this invention unless specifically designated as such. A listing of the documents is shown on enclosed Form PTO/SB/08A and copies of the foreign patents and non-patent literature are also enclosed.

The Examiner is requested to make the documents listed on the enclosed PTO/SB/08A of record in this application. Applicants would appreciate the Examiner initialing and returning the initialed copy of form PTO/SB/08A, indicating the 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-008CIPCON4.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 30 June 2020

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

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/159,282
				Filing Date	October 12, 2018
				First Named Inventor	George D. Yancopoulos
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	1	of	2	Attorney Docket Number	REGN-008CIPCON4

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				
	2				

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				
	2				

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					
	2					

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	Bayer Investor News, "VEGF Trap-Eye: New Data Confirm Successes in the Treatment of Age-related Macular Degeneration" (September 28, 2008)	
	2	Regeneron Press Release "Positive Interim Phase 2 Data Reported For VEGF Trap-Eye In Age-Related Macular Degeneration" (March 27, 2007)	
	3	Regeneron Press Release "VEGF TRAP-Eye Phase 2 Wet AMD Results Reported At Arvo Annual Meeting" (May 9, 2007)	
	4	Regeneron Press Release "Regeneron Reports Second Quarter Financial And Operating Results" (August 1, 2007)	
	5	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)	
	6	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)	
	7	Regeneron Press Release "Regeneron Reports Fourth Quarter And Full Year 2007 Financial And Operating Results" (February 27, 2008)	
	8	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)	
	9	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)	

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	16/159,282
				Filing Date	October 12, 2018
				First Named Inventor	George D. Yancopoulos
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	2	of	2	Attorney Docket Number	REGN-008CIPCON4

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
	10	Regeneron Pharmaceuticals, Inc. "Regeneron Reports Full Year and Fourth Quarter 2008 Financial and Operating Results" (February 26, 2009)	
	11	Regeneron Pharmaceuticals, Inc. "Bayer and Regeneron Extend Development Program for VEGF Trap-Eye to Include Central Retinal Vein Occlusion" (April 30, 2009)	
	12	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)	
	13	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)	
	14	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)	
	15	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)	
	16	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)	
	17	Regeneron Press Release "Regeneron And Bayer Announce Start Of Phase 3 Clinical Program In Diabetic Macular Edema" (April 8, 2011)	
	18	Regeneron Pharmaceuticals, Inc., "FDA Grants Priority Review for VEGF Trap-Eye for the Treatment of Wet Age-Related Macular Degeneration" (April 18, 2011)	
	19	Regeneron Press Release "VEGF Trap-Eye Submitted for EU Marketing Authorization for Treatment of Wet Age-Related Macular Degeneration (June 7, 2011)"	
	20	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)	
	21	Regeneron Press Release "Regeneron Announces Clinical Presentations at ASRS 2011 Annual Meeting" (August 17, 2011)	
	22	Regeneron Pharmaceuticals, Inc., "Regeneron Announces FDA Approval of EYLEA™ (aflibercept) Injection for the Treatment of Wet Age-Related Macular Degeneration: CORRECTED (November 18, 2011)	
	23	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)	
	24	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)	

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 Patent Application Fee Transmittal

Application Number:	16159282
Filing Date:	12-Oct-2018
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-008CIPCON4

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($)
Miscellaneous:				
RCE- 1ST REQUEST	1801	1	1300	1300
		Total in USD ($)		**1300**

Electronic Acknowledgement Receipt	
EFS ID:	39875398
Application Number:	16159282
International Application Number:	
Confirmation Number:	8618
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-008CIPCON4
Receipt Date:	30-JUN-2020
Filing Date:	12-OCT-2018
Time Stamp:	17:44:22
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1300
RAM confirmation Number	E20206TH44495830
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	Request for Continued Examination (RCE)	0725US05_2020-06-30_RCE_Transmittal.pdf	1352000 fbe72c4d1a7162a2001ef7e2acffdbcdcded8709	no	3
Warnings:					
Information:					
2		0725US05_2020-06-30_Pre_Amend.pdf	38519 590e8dc52c4bfe45e18b7ff08f2bab9ac8bf3398	yes	5

Multipart Description/PDF files in .zip description

Document Description	Start	End
Preliminary Amendment	1	1
Claims	2	3
Applicant Arguments/Remarks Made in an Amendment	4	5

Warnings:					
Information:					
3	Transmittal Letter	0725US05_2020-06-30_Supp_IDS_trans_REGN-008CIPCON4.pdf	50766 7cc95ccb01a190f408ac5f92e2ddbbb31794d95e	no	2
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Information:					
4	Information Disclosure Statement (IDS) Form (SB08)	0725US05__2020-06-30_Supp_IDS_SB08A_REGN-008CIPCON4.pdf	36089 5d83e275b04c9ec6f11044b9f482ac6739f27d5f	no	2
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This is not an USPTO supplied IDS fillable form					

5	Non Patent Literature	BayerNews_20080928_0448_en.pdf	127802 6d6478579d4b400aca428b8b3a577d4030031a53	no	5
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6	Non Patent Literature	REGN_Press_Release_Mar_27_2007.pdf	1360202 986d7edd7cdd3899ced99c0603f9540b36f32357	no	2
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7	Non Patent Literature	REGN_Press_Release_May_9_2007.pdf	1348579 4aea4c46411524118a56a126ad8880d5628dc9d1	no	2
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8	Non Patent Literature	REGN_Press_Release_Aug_1_2007.pdf	2839120 9d80051b30879bcf5163b4639fd18a64ca6b2bb6	no	5
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9	Non Patent Literature	REGN_Press_Release_Aug_2_2007.pdf	106077 a6c28091d1985f689be1eccf605d9cbff04cb0eb	no	2
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10	Non Patent Literature	REGN_Press_Release_Oct_1_2007.pdf	1457215 f6338613f2aabca358d8b8103489fbbf67826e36	no	3
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11	Non Patent Literature	REGN_Press_Release_Feb_27_2008.pdf	3789068 585e6714db309793c0e0f1d5dd74666c3615a379	no	6
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Information:					

12	Non Patent Literature	REGN_Press_Release_Apr_28_2008.pdf	24012 21d51e0e9eb9e2b27059dd2eaf647e2220ef6395	no	2
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13	Non Patent Literature	REGN_Press_Release_Aug_19_2008.pdf	23293 5d13932b21799c081aff58b6fa4fc0a8e6b4b825	no	2
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14	Non Patent Literature	REGN_Press_Release_Feb_26_2009.pdf	3449787 c5f9143255909 8f3ad1fb2789fde8c0fd873e91e	no	5
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15	Non Patent Literature	REGN_Press_Release_Apr_30_2009.pdf	107443 9b7019f9ac83ae628b74e4cf5d36232c548ea7e9	no	2
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16	Non Patent Literature	REGN_Press_Release_Jul_23_2009.pdf	1070186 5d38c7318355bbb4bd99263a4d5c178ce9c0eb36	no	2
Warnings:					
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17	Non Patent Literature	REGN_Press_Release_Nov_19_2010.pdf	350695 b241755a11b1083bf843f27bbf27849ece1d60a6	no	1
Warnings:					
Information:					
18	Non Patent Literature	REGN_Press_Release_Jan_18_2011.pdf	1765835 834d1807fb38ebe24b62e6859d6c78251db8b218	no	3
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19	Non Patent Literature	REGN_Press_Release_Feb_9_2011.pdf	1371797 aab4ca6426678222de6ccf81389495a08d533ce5	no	2
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Information:					
20	Non Patent Literature	REGN_Press_Release_Feb_22_2011.pdf	110830 1c15ab6a0cda244ad867d79f5839abc72511f8963	no	6
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21	Non Patent Literature	REGN_Press_Release_Apr_8_2011.pdf	1480611 a13f642ce8b29401bd55e0d61db1de29d171f413	no	2
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22	Non Patent Literature	REGN_Press_Release_Apr_18_2011.pdf	17501 a824d1d3a227ce42763b0da65601593f396c765b	no	2
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Information:					
23	Non Patent Literature	REGN_Press_Release_Jun_7_2011.pdf	1463628 41e6c89cfa55d29dff0736ab0e82cebfbec32dea	no	2
Warnings:					
Information:					
24	Non Patent Literature	REGN_Press_Release_Jun_17_2011.pdf	19498 afef5a7b39b651100449db5b31c908297f673434	no	2
Warnings:					
Information:					
25	Non Patent Literature	REGN_Press_Release_Aug_17_2011.pdf	581399 8e96e29aa8c022ae65c25ab110c11f9144593e27	no	2
Warnings:					
Information:					

26	Non Patent Literature	REGN_Press_Release_Nov_18_2011.pdf	19527 8864153c02303fe0f6969e05c4cf59b74582f9f3	no	2
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Information:					
27	Non Patent Literature	REGN_Press_Release_Nov_28_2011.pdf	27840 42379aa75f28abacc9ffca7fea7a138a3fcd374f	no	3
Warnings:					
Information:					
28	Non Patent Literature	REGN_Press_Release_Dec_5_2011.pdf	32377 cc45cb0792fb752a70ed96149f7f07e1638ad13f	no	3
Warnings:					
Information:					
29	Fee Worksheet (SB06)	fee-info.pdf	30829 1b6543069ce24d87dc4f008d806b9820ce874237	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	24452525		

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.
16/159,282	10/12/2018	George D. Yancopoulos	REGN-008CIPCON4	8618

96387 7590 07/01/2020

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
07/01/2020	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

CORRECTED ***Notice of Allowability***	**Application No.** 16/159,282	**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--

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

1. ☑ This communication is responsive to IDS filed 31 March 2020.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

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

3. ☑ The allowed claim(s) is/are 32-42 (renumbered as claims 1-11, respectively) . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

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

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

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

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

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

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

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/J.L/ Examiner, Art Unit 1647	/CHRISTINE J SAOUD/ Primary Examiner, Art Unit 1647

Notice of Pre-AIA or AIA Status

1. The present application is being examined under the pre-AIA first to invent provisions.

Information Disclosure Statement

2. The information disclosure statement (IDS) submitted on 31 March 2020 was filed after the mailing date of the Non-Final rejection on 01 October 2019. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

EXAMINER'S COMMENT

3. The information disclosure statement (IDS) filed 31 March 2020 has been considered by the Examiner. After careful consideration, the Examiner has determined that none of the information contained therein raises new issues of patentability.

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.

/Christine J Saoud/
Primary Examiner, Art Unit 1647

/J.L/
Examiner, Art Unit 1647
June 24, 2020

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/159.282
				Filing Date	October 12, 2018
				First Named Inventor	George D. Yancopoulos
				Art Unit	1647
				Examiner Name	Jon M. Lockard
Sheet	1	of	2	Attorney Docket Number	REGN-008CIPCON4

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	8092803	2012-01-10	Furfine et al.	
	3	10406226	2019-09-10	Dix et al.	
	4	10464992	2019-11-05	Furfine et al.	

U.S. PATENT APPLICATION PUBLICATIONS

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	1	2019/0388539	2019-12-26	Dix et al.	
	2	2020/0017572	2020-01-16	Furfine et al.	

FOREIGN PATENT DOCUMENTS

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	1					
	2					

NON PATENT LITERATURE DOCUMENTS

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	1	ANONYMOUS "Anti-VEGF 2019: The State of the Art" Review of Ophthalmology (published August 5, 2019)	
	2	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	
	3	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	
	4	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	
	5	CROLL et al., "VEGF-mediated inflammation precedes angiogenesis in adult brain" Experimental Neurology (2004) 187:388-402	
	6	DeVRIESE et al., "Antibodies against Vascular Endothelial Growth Factor Improve Early Renal Dysfunction in Experimental Diabetes" J. Am. Soc. Nephrol (2001) 12:993-1000	
	7	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	
	8	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	

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/

Receipt date: 03/31/2020

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	9	FERRARA, N. "Vascular Endothelial Growth Factor: Molecular and Biological Aspects" Advances in Organ Biology (1999) pp. 1-30	
	10	FERRARA et al., "Clinical applications of angiogenic growth factors and their inhibitors" Nature Medicine (December 1999) 5(12):1359-1364	
	11	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	
	12	HOLASH et al., "Vessel Cooption, Regression, and Growth in Tumors Mediated by Angiopoietins and VEGF" Science (June 18, 1999) 284(5422):1994-1998	
	13	KOROBELNIK et al., "Intravitreal Aflibercept Injection for Macular Edema Resulting from Central Retinal Vein Occlusion" American Academy of Ophthalmology (2014) 121(1):202-208	
	14	MITCHELL, Edith P. "Targeted Therapy for Metastatic Colorectal Cancer: Role of Aflibercept" Clinical Colorectal Cancer (2013) 12(2):73-85	
	15	NOGUERA-TROISE et al., "Blockade of D114 inhibits tumour growth by promoting non-productive angiogenesis" Nature (December 2006) 444:1032-1037	
	16	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	
	17	SCHMIDT-ERFURTH et al., "Intravitreal Aflibercept Injection for Neovascular Age-related Macular Degeneration" Ophthalmology (2014) 121:193-201	
	18	SEMERARO et al., "Aflibercept in wet AMD: specific role and optimal use" Drug Design, Development and Therapy (August 2, 2013) 7:711-722	
	19	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	
	20	THURSTON, Gavin "Complementary actions of VEGF and Angiopoietin-1 on blood vessel growth and leakage" J. Anat. (2002) 200:575-580	
	21	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	

Examiner Signature	/JON M LOCKARD/	Date Considered	06/24/2020

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	1	2019/0290725	2019-09-26	Vitti et al.	

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	1	WO 2004/106378 A2	2004-12-09	Regeneron Pharmaceuticals, Inc.		
	2	WO 2005/000895 A2	2005-01-05	Regeneron Pharmaceuticals, Inc.		

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	1	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)	
	2	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)	
	3	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)	
	4	HALLER et al., "VEGF Trap-Eye In CRVO: Primary Endpoint Results of the Phase 3 COPERNICUS Study" ARVO Annual Meeting Abstract (April 2011)	
	5	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)	
	6	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)	
	7	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)	
	8	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)	

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	9	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)	
	10	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)	
	11	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)	
	12	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)	
	13	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)	
	14	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)	
	15	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)	
	16	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)	
	17	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)	
	18	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)	

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	19	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)	
	20	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)	
	21	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)	
	22	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)	
	23	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)	
	24	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)	
	25	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)	
	26	Regeneron SEC Form 10-K (February 27, 2008)	
	27	Regeneron SEC Form 10-K (February 26, 2009)	
	28	Regeneron SEC Form 10-K (February 17, 2011)	
	29	Regeneron SEC Form 10-Q (May 8, 2006)	
	30	Regeneron SEC Form 10-Q (August 8, 2006)	
	31	Regeneron SEC Form 10-Q (November 6, 2006)	
	32	Regeneron SEC Form 10-Q (May 4, 2007)	
	33	Regeneron SEC Form 10-Q (August 3, 2007)	
	34	Regeneron SEC Form 10-Q (April 30, 2009)	
	35	Regeneron SEC Form 10-Q (November 3, 2009)	
	36	Regeneron SEC Form 10-Q (April 29, 2010)	
	37	Regeneron SEC Form 10-Q (July 28, 2010)	
	38	Regeneron SEC Form 10-Q (October 28, 2010)	
	39	Regeneron SEC Form 10-Q (May 3, 2011)	
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	41	Regeneron SEC Form 10-Q (October 27, 2011)	

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	42	Regeneron SEC Form 8-K Exhibit: "Press Release of Regeneron Pharmaceuticals, Inc. dated May 1, 2006" (May 2, 2006)	
	43	Regeneron SEC Form 8-K Exhibit: "Press Release of Regeneron Pharmaceuticals, Inc. dated May 3, 2006" (May 5, 2006)	
	44	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)	
	45	Regeneron SEC Form 8-K Exhibit: "Press Release dated May 2, 2007" (May 3, 2007)	
	46	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)	
	47	Regeneron SEC Form 8-K Exhibit: "Press Release dated October 1, 2007" (October 1, 2007)	
	48	Regeneron SEC Form 8-K Exhibit: "Press Release dated November 6, 2007" (November 6, 2007)	
	49	Regeneron SEC Form 8-K Exhibit: "Press Release dated May 1, 2008" (May 2, 2008)	
	50	Regeneron SEC Form 8-K Exhibit: "Press Release dated November 4, 2008" (November 4, 2008)	
	51	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)	
	52	Regeneron SEC Form 8-K Exhibit: "Press Release dated April 30, 2009" (May 1, 2009)	
	53	Regeneron SEC Form 8-K Exhibit: "Press Release dated November 3, 2009." (November 4, 2009)	
	54	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)	
	55	Regeneron SEC Form 8-K Exhibit: "Press Release dated February 17, 2011" (February 18, 2011)	
	56	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)	
	57	Regeneron SEC Form 8-K Exhibit: "Press Release dated May 3, 2011." (May 3, 2011)	
	58	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)	
	59	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)	
	60	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)	

Examiner Signature		Date Considered	

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	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)	
	62	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)	
	63	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)	
	64	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)	
	65	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)	
	66	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)	
	67	Regeneron 2008 Annual Report	
	68	Regeneron 2009 Annual Report and 10-K	
	69	Regeneron 2010 Annual Report and 10-K	
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(54) Title: VEGF TRAPS AND THERAPEUTIC USES THEREOF

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

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VEGF TRAPS AND THERAPEUTIC USES THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

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

BRIEF SUMMARY OF THE INVENTION

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

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

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

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

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

[0007] The C-region may be created in MC by insertion, deletion, or mutation, such that an enzymatically or chemically cleavable site is created. The C-region may be created in any MC and at any position within the MC; preferably, the C-region is created in a full length Fc domain, or a fragment thereof, or a C_H3 domain. The C-region may be a site cleavable by an enzyme, such as, thrombin, ficin, pepsin, matrilysin, or prolidase or cleavable chemically by, for example, formic acid or $CuCl_2$.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DETAILED DESCRIPTION OF THE INVENTION

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

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

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

General Description

[0024] The invention encompasses a VEGF trap capable of binding and inhibiting VEGF activity which is a monomer or multimer of one or more fusion polypeptides. The molecules of the invention bind and inhibit the biological action of VEGF and/or the physiological reaction or response. For a description of VEGF-receptor-based antagonist VEGF traps Flt1D2.Flk1D3.FcΔC1(a) (SEQ ID NOs:7-8) and VEGFR1R2-FcΔC1(a) (SEQ ID NOs:9-10), see PCT WO/0075319, the contents of which is incorporated in its entirety herein by reference.

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

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

Nucleic Acid Constructs and Expression

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

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

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

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

and permanently express the VEGF traps of the invention.

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

VEGF Receptor Components

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

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

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

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

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

Fusion Partner and Multimerizing Components

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

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

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

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

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

Generation of Truncated VEGF Mini-Traps

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

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

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

(SEQ ID NO:1), the dimer is exposed to thrombin under conditions which cleave one or both of the FcΔC1 domains such that truncated dimeric mini-traps are generated, having a molecular weight of approximately 50 kD – 90 kD, and has an affinity for VEGF comparable to that of the parent trap. The conditions of cleavage may be controlled by one of skill in the art to favor formation of the partial cleavage product or the fully cleaved product, the choice of cleavage conditions selected by desire for a particular product having specific properties such as molecular weight.

[0042] In a specific embodiment, the C-region is a thrombin cleavage site (LVPRGS) (SEQID NO:6) inserted into an FcΔC1 domain N-terminal to the CPPC sequence (SEQ ID NO:1). Following formation of a dimer and covalent bonding between one or both of the cysteine residues of the CPPC sequence (SEQ ID NO:1), the dimer is exposed to thrombin under conditions in which one or both of the FcΔC1 domain occur and truncated monomeric mini-traps are generated. The monomeric truncated mini-trap thus generated comprises a receptor component, and a small fragment of the Fc, and is approximately 25 kD in size and exhibits a reduced affinity for VEGF relative to the truncated dimeric trap and the full length parent trap. A similar monomeric trap produced as a recombinant protein has been shown to have a K_D of about 1 nM.

Generation of VEGF Mini-Traps

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

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

Therapetic Uses

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

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

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

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

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

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

Combination Therapies

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

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

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

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

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

Methods of Administration

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

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

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

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

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

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

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

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

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

Diagnostic and Screening Methods

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

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

Pharmaceutical Compositions

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

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

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

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

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

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

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

Cellular Transfection and Gene Therapy

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

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

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

Kits

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

Transgenic Animals

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

Specific Embodiments

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

the glycosylated polypeptides expressed in CHO cells had comparable binding affinity for VEGF as the full-sized parent trap. Example 6 further illustrates a monomeric VEGF trap consisting of $(R1R2)_2$ which is capable of binding and inhibiting VEGF. Example 7 describes the construction of a VEGF mini-trap (SEQ ID NO:26) exhibiting high affinity binding for VEGF comparable to the full length trap (SEQ ID NO:10).

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

EXAMPLES

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

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

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

Example 2: Thrombin-cleaved dimeric VEGF mini-trap

[0078] The VEGFR1R2.FcΔC1(a) (SEQ ID NOs:9-10) construct was modified by insertion of a thrombin cleavage following the CPPC (SEQ ID NO:1) of the Fc domain. Purified VEGF trap (5 μg) was incubated with thrombin (Novagen) in 20 mM Tris-HCl, pH 8.4, 50 mM NaCl, 2.5 mM $CaCl_2$ for 16 hrs at 37° C. Controls included cleavage control protein (CCP) and parent VEGF trap protein incubated without thrombin. SDS-PAGE analysis (Tris-Glycine 4-20% gel; 5 μg protein per lane) verified correct cleavage (results not shown).

[0079] Affinity determination. The Kd of binding of each VEGF trap to hVEGF165 was determined as described in WO/0075319, for the parent VEGF trap, uncleaved VEGF trap containing a thrombin cleavage site ("uncleaved VEGF trap"), cleaved VEGF mini-trap and recombinant monomeric R1R2-myc myc his. More specifically, the ability of the traps to block $VEGF_{165}$-dependent receptor phosphorylation was determined using primary human endothelial cells (HUVECs). $VEGF_{165}$ was incubated in the presence of varying concentrations of the test traps, and the mixture was added to

HUVECs to stimulate tyrosine phosphorylation of VEGFR2. At sub-stoichiometric concentrations of VEGF trap, unbound VEGF induced receptor phosphorylation. However, at a 1:1 molar ratio of greater of a VEGF trap to ligand, complete blocking of receptor signaling was observed, establishing that a single molecule of a trap dimer is capable of blocking a single molecule of human $VEGF_{165}$. Thus, the high binding affinity of the VEGF trap for VEGF results in formation of a complex that prevents VEGF from interaction with cell surface receptors. Equivalent results were obtained for identical phosphorylation inhibition experiments for the parent VEGF trap, uncleaved VEGF trap, and cleaved VEGF mini-trap The results are shown in Table 1.

TABLE 1

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

Example 3. Construction of Plasmids Encoding VEGF Mini-Traps

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

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

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

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

codon at the 5' end and fusion of the codon for cysteine, followed by a stop codon, at the 3' end (SEQ ID NO:2). This DNA fragment was then cloned into the Nco I and Not I sites of the *E. coli* expression plasmid pRG663 to yield pRG1102 such that expression of $R1R2_C$ was dependent on transcription from the phage T7 Φ1.1 promoter. Induction of gene expression from pRG1102 results in accumulation of R1R2cys in the cytoplasm of the *E. coli* host strain RFJ238. Similarly, the primers R1R2N-Nco1 (SEQ ID NO:18) and R1R2ACGC-N ot1 (SEQ ID NO:20) were used to amplify a DNA fragment from pTE115 that encodes amino acids G30 to K231 (SEQ ID NO:10) resulting in fusion of an initiating methionine codon at the 5' end and fusion of codons for ACGC (SEQ ID NO:4), followed by a stop codon, at the 3' end (SEQ ID NO:5). This fragment was then cloned into the Nco I and Not I sites of the *E. coli* expression plasmid pRG663 to yield pRG1103 such that expression of $R1R2_{ACGC}$ was dependent on transcription from the phage T7 Φ1.1 promoter. Induction of gene expression from both pRG1102 and pRG1103 resulted in accumulation of $R1R2_C$ or $R1R2_{ACGC}$, respectively, in the cytoplasm of the *E. coli* host strain RFJ238.

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

[0084] Both $R1R2_C$ and $R1R2_{ACGC}$ were expressed as cytoplasmic proteins in *E. coli* and were purified by the same method. Induction of the phage T7 Φ1.1 promoter on either pRG1102 or pRG1103 in the *E. coli* K12 strain RFJ238 resulted in accumulation of the protein in the cytoplasm. After induction, cells were collected by centrifugation, resuspended in 50 mM Tris-HCl, pH 7.5, 20 mM EDTA, and lysed by passage through a Niro-Soavi cell homogenizer. Inclusion bodies were collected from lysed cells by centrifugation, washed once in distilled H_2O, then solubilized in 8 M guanidinium-HCl, 50 mM Tris-HCl, pH 8.5, 100 mM sodium sulfite,10 mM sodium tetrathionate and incubated at room temperature for 16 hours. Clarified supernatant was fractionated on an S300 column equilibrated with 6 M guanidinium-HCl, 50 mM Tris-HCl, pH 7.5. Fractions containing $R1R2_C$ were pooled and dialyzed against 6M Urea, 50 mM Tris-HCl, pH 7.5. Dialyzed protein was diluted to 2M Urea, 50 mM Tris-HCl, pH 8.5, 2 mM cysteine then stirred slowly for 7 days at 4°C. Refolded protein was dialyzed against 50 mM Tris-HCl, pH 7.5 then loaded onto an SP-sepharose column equilibrated with 50 mM Tris-HCl, pH 7.5 and eluted with a NaCl gradient from 0 to 1 M in 50 mM Tris-HCl, pH 7.5. Fractions containing $R1R2_C$ were pooled, concentrated, and loaded onto a Superdex 200 column equilibrated with 50 mM Tris-HCl, pH 7.5, 150 mM NaCl. Fractions containing mini-trap dimer were collected and pooled. The molecular weight of purified mini-trap was estimated to be about 46 kD by SDS-PAGE.

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

TABLE 2

Trap	Kinetic Dissociation Rate (1/s)	$T_{1/2}$ (hr)
VEGF trap	4.23×10^{-5}	4.53
$R1R2_C$	3.39×10^{-5}	5.68
$R1R2_{ACGC}$	3.41×10^{-5}	5.65

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

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

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

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

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

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

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

TABLE 3

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

We claim:

1. An isolated nucleic acid molecule encoding a fusion polypeptide consisting of components $(R1R2)_X$ or $(R1R3)_Y$, and a fusion partner (FP), wherein $X \geq 1$, $Y \geq 1$, R1 is vascular endothelial cell growth factor (VEGF) receptor component Ig domain 2 of Flt-1 and R2 is Ig domain 3 of Flk-1, R3 is Ig domain 3 of Flt-4.

2. The isolated nucleic acid of claim 1, wherein the fusion partner (FP) is a multimerizing component (MC) capable of interacting with another MC to form a multimeric structure.

3. The isolated nucleic acid of claim 3, wherein the MC is selected from the group consisting of (i) a multimerizing component comprising a cleavable region (C-region), (ii) a truncated multimerizing component, (iii) an amino acid sequence between 1 to about 200 amino acids in length having at least one cysteine residue, (iv) a leucine zipper, (v) a helix loop motif, (vi) a coil-coil motif, and (vii) an immunoglobulin domain.

4. A fusion polypeptide encoded by the nucleic acid molecule of claims 1 to 3.

5. The fusion polypeptide of claim 4, having the amino acid sequence of SEQ ID NO:26, 27, or 28.

6. A replicable expression vector capable in a transformed host cell comprising the nucleic acid molecule of claims 1 to 3.

7. A method of producing a VEGF fusion polypeptide, comprising the steps of introducing into a suitable expression system the expression vector of claim 6, and effecting expression of the VEGF fusion polypeptide.

8. A vascular endothelial cell growth factor (VEGF) trap, comprising a multimer of two or more fusion polypeptides of claim 4.

9. The VEGF trap of claim 8, which is a dimer.

10. A dimeric VEGF trap comprising two fusion polypeptides comprising the amino acid sequence of SEQ ID NO:26, 27, or 28.

11. A pharmaceutical composition comprising the fusion polypeptide of claims 8 or 9, and a pharmaceutically acceptable carrier.

12. A method of treating a disease or condition which is improved, ameliorated, or inhibited by removal or inhibition of vascular endothelial growth factor (VEGF), comprising administering the pharmaceutical composition of claim 11 to a subject in need thereof.

13. The method of claim 12, wherein the disease or condition is an ocular disease or condition.

14. The method of claim 13, wherein the ocular disease or condition is age related macular degeneration.

15. An isolated nucleic acid molecule encoding a fusion polypeptide consisting of receptor components $(R1R2)_X$ or $(R1R3)_Y$, and a multimerizing component (MC) capable of interacting with another MC to form a multimeric structure, wherein $X \geq 1$, $Y \geq 1$, R1 is vascular endothelial cell growth factor (VEGF) receptor component Ig domain 2 of Flt-1 and R2 is Ig domain 3 of Flk-1, R3 is Ig domain 3 of Flt-4, wherein the multimerizing component (MC) is selected from the group consisting of (i) a MC comprising a cleavable region (C-region), (ii) a truncated MC, (iii) an amino acid sequence between 1 to about 200 amino acids in length having at least one cysteine residue, (iv) a leucine zipper, (v) a helix loop motif, (vi) a coil-coil motif, and (vii) an immunoglobulin domain.

16. The isolated nucleic acid molecule of claim 15, wherein the receptor components are $(R1R2)_X$ and the multimerizing component is an amino acid sequence between 1 to about 200 amino acids in length having at least one cysteine residue.

17. The isolated nucleic acid molecule of claim 16, wherein the receptor component is R1R2, X is 1, and the multimerizing component is an amino acid sequence 1-15 amino acids in length with 1-2 cysteine residues.

18. A fusion polypeptide capable of binding vascular endothelial growth factor (VEGF) encoded by the nucleic acid molecule of claims 15 to 17.

19. The fusion polypeptide of claim 18, comprising the amino acid sequence of SEQ ID NO:26, 27 or 28.

20. A fusion polypeptide consisting of receptor components $(R1R2)_X$ or $(R1R3)_Y$, and a multimerizing component (MC) capable of interacting with another MC to form a multimeric structure, wherein $X \geq 1$, $Y \geq 1$, R1 is vascular endothelial cell growth factor (VEGF) receptor component Ig domain 2 of Flt-1 and R2 is Ig domain 3 of Flk-1, R3 is Ig domain 3 of Flt-4, wherein the multimerizing component (MC) is selected from the group consisting of (i) a MC comprising a cleavable region (C-region), (ii) a truncated MC, (iii) an amino acid sequence between 1 to about 200 amino acids in length having at least one cysteine residue, (iv) a leucine zipper, (v) a helix loop motif, (vi) a coil-coil motif, and (vii) an immunoglobulin domain.

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

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

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

24. An article of manufacturing comprising:

(a) packaging material; and

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

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

SEQUENCE LISTING

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            260                 265                 270
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Ala Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
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Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
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Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile
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Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
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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
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            180                 185                 190
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
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    50                  55                  60
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65                  70                  75                  80
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                85                  90                  95
Gln Thr Asn Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile
            100                 105                 110
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(72) Inventors; and
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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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

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

(54) Title: METHOD OF TREATING CORNEAL TRANSPLANT REJECTION

(57) Abstract: Methods of preventing, reducing, or treating corneal transplant rejection to improve transplant survival in a subject in need thereof comprising administering an agent capable of blocking or inhibiting vascular endothelial growth factor (VEGF) are provided. The methods are useful for inhibiting or preventing corneal transplant rejection in a human subject who is the recipient of a transplanted cornea.

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METHOD OF TREATING CORNEAL TRANSPLANT REJECTION

BACKGROUND

Field of the Invention

[0001] The field of the invention is related to methods of using VEGF antagonists to reduce, prevent, or treat corneal transplant rejection, thus improving long-term transplant survival.

Description of Related Art

[0002] It has previously been reported that topical application of an anti-VEGF neutralizing antibody suppresses acute allograft rejection in a rat corneal transplant model (Yatoh et al. (1998) Transplantation 66(11):1519-24). As the leading cause of human corneal transplant failure is transplant rejection, there is a need for a therapeutic for use in preventing corneal transplant rejection in humans who receive a corneal transplant.

BRIEF SUMMARY OF THE INVENTION

[0003] The invention is based in part on the finding that administration of an agent capable of blocking or inhibiting vascular endothelial growth factor (VEGF) prevents corneal transplant rejection. The experiments, described below, conducted in an animal model of corneal transplantation show that long-term transplant survival is promoted by blocking VEGF-mediated activity.

[0004] In a first aspect, the invention features a method of improving transplant survival in a subject in need thereof, comprising administering to the subject an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity, such that transplant survival is improved.

[0005] In specific embodiments, the agent capable of blocking, inhibiting, or ameliorating VEGF-mediated activity is a VEGF antagonist. The VEGF antagonist may be a polypeptide, an antibody, a small molecule, or a nucleic acid. More specifically, the VEGF antagonist includes a VEGF trap selected from the group consisting of acetylated Flt-1(1-3)-Fc, Flt-1(1-$3_{R->N}$)-Fc, Flt-1(1-$3_{\Delta B}$)-Fc, Flt-1(2-$3_{\Delta B}$)-Fc, Flt-1(2-3)-Fc, Flt-1D2-VEGFR3D3-FcΔC1(a), Flt-1D2-Flk-1D3-FcΔC1(a), and VEGFR1R2-FcΔC1(a). In a specific and preferred embodiment, the VEGF trap is VEGFR1R2-FcΔC1(a) (also termed VEGF $trap_{R1R2}$) having the nucleotide sequence set forth in SEQ ID NO: 1 and the amino acid sequence set forth in SEQ ID NO: 2. The invention encompasses the use of a VEGF trap that is at least 90%, 95%, 98%, or at least 99%

homologous with the nucleotide sequence set forth in SEQ ID NO: 1 and/or the amino acid sequence set forth in SEQ ID NO:2.

[0006] In other embodiments, the agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity is a nucleic acid-based antagonist capable of interfering with the expression of VEGF. A specific example of this embodiment is one in which the nucleic acid-based antagonist is an aptamer, an siRNA, or an antisense molecule.

[0007] Administration of the agent may be by any method known in the art, including subcutaneous, intramuscular, intradermal, intraperitoneal, intravenous, intranasal, oral, or topical routes of administration. Preferable, administration to the subject in need of the agent is topical administration to the eye or subconjunctival administration. Administration may occur prior to or following corneal transplantation, preferably following surgery. Administration may also include a second agent, such as an immunosuppressive agent.

[0008] The subject to be treated is preferably a human subject who has or will receive a corneal transplant.

[0009] In a related second aspect, the invention features the use of an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity in the preparation of a medicament for improving transplant survival in a mammalian subject.

[0010] In a third aspect, the invention features a method of preventing corneal transplant rejection in a subject in need thereof, comprising administering to the subject an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity, such that corneal transplant rejection is prevented.

[0011] In a related fourth aspect, the invention features the use of an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity in the preparation of a medicament for the treatment of corneal transplant rejection in a mammalian subject.

[0012] In a fifth aspect, the invention features a method of reducing the incidence of corneal transplant rejection in a subject in need thereof, comprising administering to the subject an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity, such that the incidence of corneal transplant rejection is reduced.

[0013] In a related sixth aspect, the invention features the use of an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity in the preparation of a medicament for reducing the incidence of corneal transplant rejection in a mammalian subject receiving a corneal transplant.

[0014] In a seventh aspect, the invention features a pharmaceutical composition comprising a VEGF antagonist, for example the VEGF trap VEGFR1R2-FcΔC1(a), in a pharmaceutically

acceptable carrier. Such pharmaceutical compositions may be liquid, gel, ointment, salve, slow release formulations or other formulations suitable for ophthalmic administration.

[0015] In an eighth aspect, the invention features an article of manufacture comprising packaging materials and a pharmaceutical agent contained within the packaging materials, wherein the pharmaceutical agent comprises at least one VEGF-specific fusion protein of the invention, and the packaging material comprises a label or package insert which indicates that the VEGF-specific fusion protein can be used for the treatment or prevention of corneal transplant rejection.

[0016] Other objects and advantages will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION

[0017] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0018] As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0019] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

General Description

[0020] Experiments were undertaken to evaluate occurrence and time course of hem- and lymphangiogenesis after normal-risk corneal transplantation and to test whether pharmacologic strategies inhibiting both processes improve long-term graft survival. As described in the experimental section below, normal-risk allogeneic (C57BL/6 to BALB/c) and syngeneic (BALB/c to BALB/c) corneal transplantations were performed and occurrence and time course

of hem- and lymphangiogenesis after keratoplasty was observed using double immunofluorescence of corneal flatmounts (with CD31 as panendothelial and LYVE-1 as lymphatic vascular endothelial specific marker). A molecular trap designed to eliminate VEGF-A ("VEGF $Trap_{R1R2}$"; 12.5 mg/kg) was tested for its ability to inhibit both processes after keratoplasty and to promote long-term graft survival (intraperitoneal injections on the day of surgery and 3, 7, and 14 days later). The results show that no blood or lymph vessels were detectable immediately after normal-risk transplantation in either donor or host cornea, but hem- and lymphangiogenesis were clearly visible at day 3 after transplantation. Both vessel types reached donor tissue at one week after allo- and similarly after syngeneic grafting. Early postoperative trapping of VEGF-A significantly reduced both hem- and lymphangiogenesis and significantly improved long-term graft survival (78% versus 40%; $p<0.05$). There is concurrent, VEGF-A-dependent hem- and lymphangiogenesis after normal-risk keratoplasty within the preoperatively avascular recipient bed. Inhibition of hem- and lymphangiogenesis (which mediate the efferent and afferent arms of an immune response) after normal-risk corneal transplantation improves long-term graft survival, establishing that early postoperative hem- and lymphangiogenesis are risk factors for graft rejection even in low-risk eyes.

Definitions

[0021] By the term "therapeutically effective dose" is meant a dose that produces the desired effect for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

[0022] By the term "blocker", "inhibitor", or "antagonist" is meant a substance that retards or prevents a chemical or physiological reaction or response. Common blockers or inhibitors include but are not limited to antisense molecules, antibodies, antagonists and their derivatives. More specifically, an example of a VEGF blocker or inhibitor is a VEGF receptor-based antagonist including, for example, an anti-VEGF antibody, or a VEGF trap such as VEGFR1R2-FcΔC1(a) (SEQ ID NOs:1-2). For a complete description of VEGF-receptor based antagonists including VEGFR1R2-FcΔC1(a), see PCT publication WO/00/75319, the contents of which is incorporated in its entirety herein by reference.

[0023] A "small molecule" is defined herein to have a molecular weight below about 500 Daltons, and may include chemical as well as peptide molecules.

VEGF Antagonists

[0024] In one aspect of the invention, VEGF-mediated activity is blocked or inhibited by the use

of VEGF receptor-based blockers of VEGF-mediated activity. A non-limiting example of a VEGF receptor-based blocker includes, but is not limited to, VEGFR1R2-FcΔC1(a). Other suitable receptor-based blockers include acetylated Flt-1(1-3)-Fc, Flt-1(1-$3_{R->N}$)-Fc, Flt-1(1-$3_{\Delta B}$)-Fc, Flt-1(2-$3_{\Delta B}$)-Fc, Flt-1(2-3)-Fc, Flt-1D2-VEGFR3D3-FcΔC1(a), Flt-1D2-Flk-1D3-FcΔC1(a). For a complete description of these and other VEGF-receptor-based blockers, including pegylated receptor-based blockers, see PCT Publication No. WO/00/75319, the contents of which is incorporated in its entirety herein by reference.

[0025] In addition to the VEGF receptor-based blockers described in PCT Publication No. WO/00/75319, variants and derivatives of such VEGF receptor-based blockers are also contemplated by the invention. The sequence of the variants or derivatives may differ by a change which is one or more additions, insertions, deletions and/or substitutions of one or more nucleotides of the sequence set forth in SEQ ID NO:1. Changes to a nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the genetic code. Thus, nucleic acid according to the present invention may include a sequence different from the sequence shown in SEQ ID NO:1, yet encode a polypeptide with the same amino acid sequence as SEQ ID NO: 2. On the other hand, the encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequence shown in SEQ ID NO:2. Nucleic acid encoding a polypeptide which is an amino acid sequence variant or derivative of the sequence shown in SEQ ID NO:2 is further provided by the present invention. Nucleic acid encoding such a polypeptide may show at the nucleotide sequence and/or encoded amino acid level greater than about 90%, 95%, 98%, or 99% homology with the coding sequence shown in SEQ ID NO:1 and/or the amino acid sequence shown in SEQ ID NO:2. For amino acid "homology", this may be understood to be similarity (according to the established principles of amino acid similarity, e.g. as determined using the algorithm GAP (Genetics Computer Group, Madison, Wis.)) or identity. GAP uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, the default parameters are used, with a gap creation penalty=12 and gap extension penalty=4.

[0026] Individual components of the VEGF-specific fusion proteins of the invention may be constructed by molecular biological methods known to the art with the instructions provided by the instant specification. These components are selected from a first cellular receptor protein, such as, for example, VEGFR1; a second cellular receptor protein, such as, for example, VEGFR2; a multimerizing component, such as an Fc.

[0027] Specific embodiments of the VEGF-specific fusion proteins useful in the methods of the invention comprise a multimerizing component which allows the fusion proteins to associate,

e.g., as multimers, preferably dimers. Preferably, the multimerizing component comprises an immunoglobulin derived domain. Suitable multimerizing components are sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al. 1982 Cell 29:671-679); immunoglobulin gene sequences, and portions thereof.

[0028] The nucleic acid constructs encoding the fusion proteins useful in the methods of the invention are inserted into an expression vector by methods known to the art, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Host-vector systems for the production of proteins comprising an expression vector introduced into a host cell suitable for expression of the protein are known in the art. The suitable host cell may be a bacterial cell such as *E. coli*, a yeast cell, such as *Pichia pastoris,* an insect cell, such as *Spodoptera frugiperda*, or a mammalian cell, such as a COS, CHO, 293, BHK or NS0 cell.

Antisense Nucleic Acids

[0029] In one aspect of the invention, VEGF-mediated activity is blocked or inhibited by the use of VEGF antisense nucleic acids. The present invention provides the therapeutic or prophylactic use of nucleic acids comprising at least six nucleotides that are antisense to a gene or cDNA encoding VEGF or a portion thereof. As used herein, a VEGF "antisense" nucleic acid refers to a nucleic acid capable of hybridizing by virtue of some sequence complementarity to a portion of an RNA (preferably mRNA) encoding VEGF. The antisense nucleic acid may be complementary to a coding and/or noncoding region of an mRNA encoding VEGF. Such antisense nucleic acids have utility as compounds that prevent VEGF expression, and can be used in the treatment or prevention of corneal transplant rejection. The antisense nucleic acids of the invention are double-stranded or single-stranded oligonucleotides, RNA or DNA or a modification or derivative thereof, and can be directly administered to a cell or produced intracellularly by transcription of exogenous, introduced sequences.

[0028] The VEGF antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides ranging from 6 to about 50 oligonucleotides. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof and can be single-stranded or double-stranded. In addition, the antisense molecules may be polymers that are nucleic acid mimics, such as PNA, morpholino oligos, and LNA. Other types of antisence molecules include short double-stranded RNAs, known as siRNAs, and short hairpin RNAs, and long dsRNA (>50 bp but usually ≥500 bp).

Short interfering RNAs

[0029] In another embodiment, VEGF-mediated activity is blocked by blocking VEGF expression. One method for inhibiting VEGF expression is the use of short interfering RNA (siRNA) through RNA interference (RNAi) or post-transcriptional gene silencing (PTGS) (see, for example, Ketting et al. (2001) Genes Develop. 15:2654-2659). siRNA molecules can target homologous mRNA molecules for destruction by cleaving the mRNA molecule within the region spanned by the siRNA molecule. Accordingly, siRNAs capable of targeting and cleaving homologous VEGF mRNA are useful for treating, reducing or preventing corneal transplant rejection.

Inhibitory Ribozymes

[0030] In aspect of the invention, corneal transplant rejection may be treated or prevented in a subject suffering from such disease by decreasing the level of VEGF activity by using ribozyme molecules designed to catalytically cleave gene mRNA transcripts encoding VEGF, preventing translation of target gene mRNA and, therefore, expression of the gene product.

[0031] Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, *e.g.*, U.S. Patent No. 5,093,246. While ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy mRNAs encoding VEGF, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA has the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art. The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA). The Cech-type ribozymes have an eight base pair active site that hybridizes to a target RNA sequence where after cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes that target eight base-pair active site sequences that are present in the gene encoding VEGF.

Generation of Antibodies to VEGF Proteins

[0032] In another aspect of the invention, the invention may be practiced with an anti-VEGF

antibody or antibody fragment capable of binding and blocking VEGF activity. Anti-VEGF antibodies are disclosed, for example, in US Patent No. 6,121,230, herein specifically incorporated by reference. The term "antibody" as used herein refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda , alpha, gamma, delta, epsilon, and mu constant regions, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively. Within each IgG class, there are different isotypes (eg. IgG_1, IgG_2, etc.). Typically, the antigen-binding region of an antibody will be the most critical in determining specificity and affinity of binding.

[0033] Antibodies exist as intact immunoglobulins, or as a number of well-characterized fragments produced by digestion with various peptidases. For example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region. While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the terms antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (e.g., single chain Fv)(scFv) or those identified using phase display libraries (see, for example, McCafferty et al. (1990) Nature 348:552-554).

[0034] Methods for preparing antibodies are known to the art. See, for example, Kohler & Milstein (1975) Nature 256:495-497; Harlow & Lane (1988) Antibodies: a Laboratory Manual, Cold Spring Harbor Lab., Cold Spring Harbor, NY). The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, e.g., the genes encoding a monoclonal antibody can be cloned from a hybridoma and used to produce a recombinant monoclonal antibody. Gene libraries encoding heavy and light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic specificity. Techniques for the production of single chain antibodies or recombinant antibodies (US 4,946,778; US 4,816,567) can be adapted to produce antibodies used in the fusion proteins and methods of the instant invention. Also, transgenic mice, or other organisms such as other mammals, may be

used to express human or humanized antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens.

Antibody Screening and Selection

[0035] Screening and selection of preferred antibodies can be conducted by a variety of methods known to the art. Initial screening for the presence of monoclonal antibodies specific to a target antigen may be conducted through the use of ELISA-based methods, for example. A secondary screen is preferably conducted to identify and select a desired monoclonal antibody for use in construction of the multi-specific fusion proteins of the invention. Secondary screening may be conducted with any suitable method known to the art. One preferred method, termed "Biosensor Modification-Assisted Profiling" ("BiaMAP") is described in co-pending USSN 60/423,017 filed 01 Nov 2002, herein specifically incorporated by reference in its entirety. BiaMAP allows rapid identification of hybridoma clones producing monoclonal antibodies with desired characteristics. More specifically, monoclonal antibodies are sorted into distinct epitope-related groups based on evaluation of antibody:antigen interactions.

Treatment Population

[0036] A suitable subject for treatment by the method of the invention is a human who has received or will receive a corneal transplant. Corneal transplantation is the oldest, most successful and most commonly performed tissue transplantation, with nearly 40,000 transplantations a year alone in the US. When corneal grafts are placed into an avascular recipient bed (so-called normal-risk keratoplasty), 2-year graft survival rates approach 90% under cover of topical steroids, even without HLA-matching. This very successful outcome is attributed to corneal immune privilege, i.e. the phenomenon of suppressed corneal inflammation induced by an array of endogenous mechanisms downregulating alloimmune and inflammatory responses in the cornea and its bed. These mechanisms include the lack of both afferent lymphatic and efferent blood vessels in the normal-risk recipient cornea, lack of MHC II^+ antigen presenting cells (APCs), FASL-expression on corneal epithelium and endothelium, and the anterior chamber associated immune privilege (ACAID) directed at graft antigens etc. (Streilein et al. (1999) Transplant Proc. 31:1472-1475).

[0037] In contrast, survival rates of cornea grafts placed into vascularized, not immune-privileged recipient beds (so called high-risk keratoplasty) drop significantly to below 50% (even with local and systemic immune suppression). Pre-existing corneal stromal blood vessels have been identified as strong risk factors for immune rejection after corneal transplantation, both in

the clinical setting as well as in the well-defined mouse model of corneal transplantation (Sano et al. (1995) Invest. Ophthalmol. Vis. Sci. 36:2176-85). Recently, in addition to blood vessels, biomicroscopically undetectable lymphatic vessels have been found in association with blood vessels in vascularized high-risk human corneas (Cursiefen et al. (2003) Cornea. 22:273-81) and it is likely that corneal lymphatic vessels enable effective access of donor and host APCs and antigenic material to regional lymph nodes where accelerated sensitisation to graft antigens occurs (Liu et al. (2002) J. Exp. Med. 195:259-68) even in the normal-risk setting (with a preoperatively avascular recipient bed), where mild corneal hemangiogenesis develops after keratoplasty. Outgrowth of new blood vessels from the limbal arcade towards the graft can be observed within the first postoperative year in about 50% of patients undergoing normal-risk keratoplasty, and in 10% of patients these new blood vessels even reach the interface or invade donor tissue (Cursiefen et al. (2001) Graefes Arch. clin. Exp. Ophthalmol. 39:514-21) at corneal suture sites, and then proceed centrally.

Methods of Administration

[0038] The invention provides methods of treatment comprising administering to a subject an effective amount of an agent of the invention. In a preferred aspect, the agent is substantially purified (*e.g.*, substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, *e.g.*, such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[0039] Various delivery systems are known and can be used to administer an active agent of the invention, *e.g.*, delivery systems suitable for topical administration, preferably topical administration directly to the eye, or subconjunctival administration, as well as other delivery systems such as those that utilize encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction are preferably topical or subconjunctival, but may be enteral or parenteral including but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, and oral routes. The active agents may be administered by any convenient route, for example by absorption through epithelial (e.g. topical administration to the eye) or mucocutaneous linings (*e.g.*, oral mucosa, intestinal mucosa, etc.) or infusion or bolus injection, and may be administered together with other biologically active agents. Administration can be systemic or local. Administration can be acute or chronic (*e.g.* daily, weekly, monthly, etc.) or in combination or alteration with other agents. Pulmonary

administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0040] In another embodiment, the active agent can be delivered in a vesicle, in particular a liposome (see Langer (1990) Science 249:1527-1533). In yet another embodiment, the active agent can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer (1990) *supra*). In another embodiment, polymeric materials can be used (see Howard et al. (1989) J. Neurosurg. 71:105). In another embodiment where the active agent of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see, for example, U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0041] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by topical administration, subconjunctival administration, local infusion during surgery, *e.g.*, by injection, by means of a catheter, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, fibers, or commercial skin substitutes.

Cellular Transfection and Gene Therapy

[0042] The present invention encompasses the use of nucleic acids encoding the VEGF-specific fusion proteins of the invention for transfection of cells *in vitro* and *in vivo*. These nucleic acids can be inserted into any of a number of well-known vectors for transfection of target cells and organisms. The nucleic acids are transfected into cells *ex vivo* and *in vivo*, through the interaction of the vector and the target cell. Reintroduction of transfected cells may be accomplished by any method known to the art, including re-implantation of encapsulated cells. The compositions are administered (e.g., by injection into a muscle) to a subject in an amount sufficient to elicit a therapeutic response. An amount adequate to accomplish this is defined as "a therapeutically effective dose or amount."

[0043] In another aspect, the invention provides a method of treating or preventing corneal transplant rejection in a human comprising transfecting a cell with a nucleic acid encoding a

VEGF-specific fusion protein of the invention, wherein the nucleic acid comprises an inducible promoter operably linked to the nucleic acid encoding the VEGF-specific fusion protein. For gene therapy procedures in the treatment or prevention of human disease, see for example, Van Brunt (1998) Biotechnology 6:1149-1154.

Pharmaceutical Compositions

[0044] Pharmaceutical compositions useful in the practice of the method of the invention include a therapeutically effective amount of an active agent, and a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

[0045] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, or intramuscular administration to human beings. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0046] The active agents of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those

derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0047] The amount of the active agent of the invention that will be effective in the treatment or prevention of corneal transplant rejeciton can be determined by standard clinical techniques based on the present description. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each subject's circumstances. However, suitable dosage ranges for intravenous administration are generally about 50-5000 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0048] For systemic administration, a therapeutically effective dose can be estimated initially from *in vitro* assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Initial dosages can also be estimated from *in vivo* data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

[0049] Dosage amount and interval may be adjusted individually to provide plasma levels of the compounds that are sufficient to maintain therapeutic effect. One having skill in the art will be able to optimize therapeutically effective local dosages witho ut undue experimentation.

[0050] The amount of compound administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration, and the judgment of the prescribing physician. The therapy may be repeated intermittently while symptoms are detectable or even when they are not detectable. The therapy may be provided alone or in combination with other drugs.

Combination Therapies

[0051] In numerous embodiments, the VEGF blockers of the present invention may be administered in combination with one or more additional compounds or therapies or medical procedures. For example, suitable therapeutic agents for use in combination, either alternating or simultaneously, with the VEGF blockers may include topically administered immunosuppressive

agents such as corticosteroids, dexamethasone, cyclosporin A, or anti-metabolic agents or systemically administered immunosuppressive agents such as corticosteroids, dexamethasone, cyclosporin A, FK506, or anti-metabolic agents, as well as other agents effective to treat, reduce, or prevent corneal transplant rejection (see Barker, NH, *et al.*, (2000) Clin Exp Opthal 28:357-360). Other suitable therapeutic agents for use in combination, either alternating or simultaneously, with the VEGF blockers of the subject invention may include blockers that can block other VEGF family members such as VEGF-C and VEGF-D.

Kits

[0052] The invention also provides an article of manufacturing comprising packaging material and a pharmaceutical agent contained within the packaging material, wherein the pharmaceutical agent comprises at least one VEGF-specific fusion protein of the invention and wherein the packaging material comprises a label or package insert which indicates that the VEGF-specific fusion protein can be used for treating corneal transplant rejection.

[0053] Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

EXAMPLES

[0054] The following example is put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Inhibition of corneal lymphangiogenesis and angiogenesis after low-risk keratoplasty using VEGFR1R2-FcΔC1(a).

[0055] Mice and anesthesia. Six to 8 weeks old male C57BL/6 mice were used as donors and same-aged male BALB/c mice (Taconic, Germantown, NY) as recipients in the mouse model of normal-risk keratoplasty (Sonoda et al. (1992) Transplantation 54:694-704). For syngeneic transplantations, 6-8 weeks old male BALB/c mice were used both as donors as well as recipients. For the dose response studies, 8 weeks old male C57BL/6 mice were used. All animals were treated in accordance with the ARVO Statement for the Use of Animals in

Ophthalmic and Vision Research. Mice were anesthetized using a mixture of ketamine and xylazine (120 mg/kg body weight and 20 mg/kg body weight respectively).

[0056] Dose response of VEGF Trap$_{R1R2}$. Five different doses of VEGF-Trap$_{R1R2}$ (SEQ ID NO:2) were tested in mice that received three interrupted intrastromal sutures (10-0 nylon, 50-μm-diameter, Sharpoint, Surgical Specialties Corporation, Reading, PA). Gentamicine and ophthalmic ointment were applied immediately after surgery. Following surgery (day 0), mice received a single subcutaneous injection of VEGF Trap$_{R1R2}$ (25 mg/kg, 12.5 mg/kg, 6.25 mg/kg, 2.5 mg/kg or 0.5 mg) or human Fc (12.5 mg/kg; control). Corneas were harvested on day 9 after suture placement, following an intravenous administration of an endothelial-specific fluorescein-conjugated lectin (*Lycopersicon esculentum*, Vector Laboratories, Burlingame, CA). The isolated corneas were flat-mounted on glass slides, and images of lectin-labeled vessels were captured using a Spot RT Digital camera (Diagnostic Instrument, Inc. Sterling Heights, MI) attached to a Nikon Microphot-FXA microscope (Nikon Inc. Garden City, NY). Scion Image 1.62c (Scion Corporation, Frederick, MD) was used to quantify the extent of corneal neovascularization.

[0057] Corneal transplantation in mice. Orthotopic corneal allografting in the mouse model of normal-risk keratoplasty was performed as described previously (Sonoda et al. (1992) *supra*). Donor corneas were excised by trephination using a 2.0 mm bore and cut with a curved vannas scissor. Until grafting, corneal tissue was placed in chilled phosphate-buffered saline. Recipients were anesthetized and the graft bed was prepared by trephining a 1.5 mm site in the central cornea of the right eye and discarding the excised cornea. The donor cornea was immediately applied to the bed and secured in place with 8 interrupted sutures (11-0 nylon, 70 μm diameter needles, Arosurgical, Newport Beach, CA). Antibiotic ointment (Oxymycin, Pharmafair, Hauppauge, NY) was placed on the corneal surface and the eyelids sutured with 8-0 suture (Sharpoint, Reading, PA). Recipients of grafts in which bleeding developed in the immediate postoperative period were discarded from further evaluation. All grafted eyes were examined after 72 hours, and grafts with technical difficulties (hyphema, cataract, infection, loss of anterior chamber) were excluded from further consideration. Tarsorraphy and corneal sutures were removed after 7 days and grafts were then examined at least twice a week until week 8 post transplantation by slit-lamp microscopy and scored for opacity. The survival experiment was performed twice and comprised 10 and 12 mice per experiment in both groups, respectively. Clinical scores of corneal grafts for opacity were as follows: 0= clear; +1=minimal, superficial (nonstromal) opacity; pupil margin and iris vessels readily visible through the cornea; +2= minimal, deep (stroma) opacity; pupil margins and iris vessels visible; +3= moderate stromal opacity; only pupil margin visible; +4= intense stromal opacity; only a portion of pupil margin

visible; +5= maximum stromal opacity; anterior chamber not visible. Grafts with opacity scores of +2 or greater after 2 weeks were considered to have been rejected. Syngeneic transplantations were performed and evaluated in a similar manner.

[0058] Immunohistochemistry and morphometry of angiogenesis and lymphangiogenesis in the cornea. Briefly, corneal flat mounts were rinsed in PBS, fixed in acetone, rinsed in PBS, blocked in 2% bovine serum albumin, stained with FITC-conjugated CD31/PECAM-1 overnight (Santa Cruz Biotechnology, Santa Cruz, CA; 1:100), washed, blocked, stained with LYVE-1 (1:500; a lymphatic endothelium specific hyaluronic acid receptor (Cursiefen et al. (2002) Invest. Ophthalmol. Vis. Sci. 43:2127-35) washed, blocked, and stained with Cy3 (1:100; Jackson ImunoResearch Laboratories, West Grove, PA) and analyzed using a Zeiss Axiophot microscope. Digital pictures of the flat mounts were taken using Spot Image Analysis system. Then the area covered by $CD31^{+++}$/LYVE-1^{-} blood vessels and $CD31^{+}$/LYVE-1^{+++} lymph vessels was measured morphometrically on these flat-mounts using NIH Image software. The total corneal area was outlined using the innermost vessel of the limbal arcade as the border. The total area of blood versus lymphatic neovascularization was then normalized to the total corneal area and the percentage of the cornea covered by each vessel type calculated.

[0059] Neutralization of VEGF-A using VEGF $Trap_{R1R2}$. The VEGF $trap_{R1R2}$ (Regeneron Pharmaceuticals Inc, Tarrytown, NY (Holash et al. (2002) Proc. Natl. Acad. Sci. USA 99:11393-8, herein specifically incorporated by reference in its entirety) was used in the transplant survival experiment at a concentration of 12.5 mg/kg intraperitoneally (i.p.) at time of surgery (CHO hVEGFR1 [Ig domain 2] R2 [Ig domain 3]-Fc), and 3, 7, and 14 days after surgery. Human Fc-fragment given i.p. at same concentration and times was used in the control mice (sCHO h Fc).

[0060] Statistical analysis. Statistical significance was analyzed by Mann-Whitney's test. Differences were considered significant at $P < 0.05$. Each experiment was performed at least twice with similar results. Graphs were drawn using Graph Pad Prism, Version 3.02.

[0061] <u>Results.</u> Dose response of angiogenesis inhibition by VEGF $Trap_{R1R2}$. VEGF-$Trap_{R1R2}$ at doses of either 25 mg/kg or 12.5 mg/kg completely inhibited suture-induced inflammatory corneal neovascularization. In contrast, doses of 6.25mg/kg and 2.5mg/kg produced ~50% and ~20% inhibition of corneal neovascularization, respectively, while the lowest dose tested, 0.5 mg/kg, had a negligible effect (<5% inhibition). Therefore, for subsequent experiments a dose of 12.5 mg/kg VEGF $Trap_{R1R2}$ was chosen.

[0062] Rapid and parallel onset of hemangiogenesis and lymphangiogenesis *after* normal-risk allogeneic corneal transplantation. To determine whether the mild and temporary hemangiogenesis occurring *after* normal-risk keratoplasty is accompanied by lymphatic vessel outgrowth from the limbus into the normally alymphatic cornea, we studied the time course of

ingrowth of both vessel types at days 0, 3, 7, 14, 21, and 28 *after* allogeneic keratoplasty (only accepted grafts). Immediately *after* surgery, blood and lymphatic vessels were not detectable either in the host or in donor tissue using biomicroscopy and immunohistochemistry on corneal flat mounts. But, at day 3 after allografting, both methods revealed new blood vessels growing into the cornea already 1/3 to halfway towards the graft interface. By day 7 these vessels had usually reached the donor tissue, but they rarely invaded the donor tissue itself. Analyzing flatmounts stained with LYVE-1 as a lymphatic vessel specific marker showed that $CD31^{+++}$/LYVE-1^{-} blood vessels were regularly accompanied by LYVE-1^{+++}/$CD31^{+}$ lymphatic vessels. Both vessel types reached the interface simultaneously at day 7. Thereafter, coincident with suture removal, both vessel types started to regress (if no immune rejection occurred; data not shown).

[0063] No difference in postkeratoplasty hem- and lymphangiogenesis between syngeneic and allogeneic corneal transplantation. To determine whether the simultaneous induction of hem- and lymphangiogenesis *after* normal-risk keratoplasty is primarily an effect of the surgical trauma, suturing and wound healing processes or secondary to early immunological rejection reactions, we compared speed and extent of both hem- and lymphangiogenesis occurring *after* keratoplasty between allogeneic (C57BL/6 into BALB/c) and syngeneic grafts (BALB/c into BALB/c) at day 3, 7, 14, 21, 28 after transplantation. In both groups, blood and lymphatic vessels grew out after keratoplasty and by day 3 reached about 1/3 to _ of the limbus-interface distance. At day 7 after syngeneic and allogeneic grafting both vessel types had reached the interface, before they started to regress thereafter. Furthermore, there was no significant difference in the hem- and lymphvascularized area, comparing syngeneic and allogeneic grafts at 3 days (allogeneic: hemvascularized area [HA] 25.2±4.1% and lymphvascularized area [LA] 22.2±9.4% versus syngeneic HA: 23±2.7% and LA 19.4±7.2%) and 7 days (allogeneic HA: 53.8±11.2% and LA: 37.9±6.2% versus syngeneic HA: 55.9±8.2% and LA: 38±22.7%) after surgery (n=8 mice per group per timepoint).

[0064] Neutralization of VEGF-A after normal-risk keratoplasty inhibits postoperative hemangiogenesis and lymphangiogenesis. Mice received either intraperitoneal injections of VEGF $Trap_{R1R2}$ (12.5 mg/kg) at surgery and 3 days later, or in the controls the Fc-protein in the same dosage. At day 3 and 7 after surgery, the extent of hem- and lymphangiogenesis was compared between these two groups (n=6 mice per group per timepoint). At day 3 and day 7 after surgery, the hemvascularized area was significantly smaller in trap-treated mice (day 3: 15.8±4.0%; day 7: 25.2±13.3%) compared to mice just receiving the Fc-fragment (day 3: 25.8±4.4%; day 7: 48.3±12.8%; $p<0.0001$). This was also true for the lymphvascularized area

comparing Trap- (9.5±9.4%) and Fc-treated mice on day 3 (21.5±9.3%; p<0.0001). At day 7, the lymphvascularized area was smaller, but not significantly different in the Trap-group (28.7±20.3%) compared to the Fc-group (51.5±23.8%; p=0.06). In contrast to results obtained in corneal injury models neither hem- or lymphangiogenesis were completely inhibited by the VEGF $Trap_{R1R2}$ following corneal transplantation. However, the number of lymphatic vessels reaching the graft-host interface (10.6±0.6 versus 1.3±1.5 vessels) and the number of hours where the interface was filled with draining lymphatic vessels were much larger in the Fc-treated compared to the Trap-treated group (3±2 versus 0.2±0.3 hours; not significant due to small sample size) at day 7. This might indicate that lymphavascularized area per se is less decisive for host sensitisation than the contact area with donor tissue.

[0065] Partial inhibition of early postoperative hem- and lymphangiogenesis by trapping VEGF-A after normal-risk surgery improves long-term graft survival.

Since hem- and lymphangiogenesis occurring *after* normal-risk keratoplasty peaked around day 7, and regressed thereafter, and since both vascular processes could be significantly inhibited by early postoperative neutralization of VEGF-A, we determined whether inhibition of postkeratoplasty hem- and lymphangiogenesis during this interval improves graft survival. The long-term survival of C57BL/6 grafts placed into avascular BALB/c recipient beds was compared between mice receiving an i.p. injection of 12.5 mg/kg VEGF $Trap_{R1R2}$, or Fc-fragment alone, at surgery and 3, 7, and 14 days later. Trapping of VEGF-A postoperatively caused a significantly improved long-term graft survival at 8 weeks (78%), compared to grafts in eyes of Fc-treated controls (40%; p=0.044; n=22 in both groups).

[0066] The present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof.

Claims

We claim,

1. Use of an first agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity in the preparation of a medicament for treating or preventing corneal transplant rejection in a mammalian subject.

2. The use of claim 1, wherein the agent capable of blocking, inhibiting, or ameliorating VEGF-mediated activity is a VEGF antagonist.

3. The use of claim 2, wherein the VEGF antagonist is a polypeptide, an antibody, a small molecule, or a nucleic acid.

4. The use of claim 3, wherein the VEGF antagonist includes a VEGF trap selected from the group consisting of acetylated Flt-1(1-3)-Fc, Flt-1(1-$3_{R->N}$)-Fc, Flt-1(1-$3_{\Delta B}$)-Fc, Flt-1(2-$3_{\Delta B}$)-Fc, Flt-1(2-3)-Fc, Flt-1D2-VEGFR3D3-FcΔC1(a), Flt-1D2-Flk-1D3-FcΔC1(a), and VEGFR1R2-FcΔC1(a).

5. The use of claim 4, wherein the VEGF trap is VEGFR1R2-FcΔC1(a).

6. The use of claim 3, wherein the VEGF antagonist is a nucleic acid selected from the group consisting of aptamer, an siRNA, or an antisense molecule.

7. The use of claim 1, wherein administration is subcutaneous, intramuscular, intradermal, intraperitoneal, intravenous, intranasal, oral, subconjunctival, or topical. Administration may also include a second agent, such as an immunosuppressive agent.

8. The use of claim 1, further comprising administering a second agent.

9. The use of claim 8, wherein the second agent is an immunosuppressive agent.

10. The use of claim 1, wherein the mammalian subject is a human.

11. The use of claim 10, wherein the human subject has received a corneal transplant.

12. A method of reducing the incidence of corneal transplant rejection in a subject in need thereof, comprising administering to the subject an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity, such that the incidence of corneal transplant rejection is reduced.

13. A method of treating corneal transplant rejection in a subject in need thereof, comprising administering to the subject an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity, such that corneal transplant rejection is treated.

14. A pharmaceutical composition for prevention or treatment of corneal transplant rejection, comprising a vascular endothelial growth factor (VEGF) antagonist, and a pharmaceutically acceptable carrier.

15. The pharmaceutical composition of claim 14, in the form of a liquid, gel, ointment, salve, or ophthalmic solution.

16. An article of manufacturing comprising:

(a) packaging material; and

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

wherein the pharmaceutical agent comprises at least one VEGF-specific fusion protein of the invention and wherein the packaging material comprises a label or package insert which indicatess that the VEGF-specific fusion protein can be used to treat or prevent corneal transplant rejection in a mammalian subject.

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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-008CIPCON4
Receipt Date:	16-JUL-2020
Filing Date:	12-OCT-2018
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Application Type:	Utility under 35 USC 111(a)

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35	Non Patent Literature	31_20061106_REGENERON_PHARMACEUTICALS_INC_10-Q_11_6.pdf	2058625 f68aeb73dbb29d2f29ef97c6ea5a6cfeb3c5106a	no	174
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36	Non Patent Literature	32_20070504_REGENERON_PHARMACEUTICALS_INC_10-Q_5_4.pdf	1163823 2abbef802132edbc86b12510f49f8f81ccba7cb4	no	92
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Information:					

37	Non Patent Literature	33_20070803_REGENERON_PHARMACEUTICALS_INC_10-Q_8_3.pdf	938799 f19ec4b03013f5f3b41424887f37672ad2e26331	no	66
Warnings:					
Information:					
38	Non Patent Literature	34_20090430_REGENERON_PHARMACEUTICALS_INC_10-Q_4_30.pdf	1262748 64632b48b21ea7789c60d93cd35101c4149910c9	no	87
Warnings:					
Information:					
39	Non Patent Literature	35_20091103_REGENERON_PHARMACEUTICALS_INC_10-Q_11_3.pdf	1103949 aa3c296cb48c93420a9a1790b221d111a815e2d0	no	68
Warnings:					
Information:					
40	Non Patent Literature	36_20100429_REGENERON_PHARMACEUTICALS_INC_10-Q_4_29.pdf	936521 aa50813d2bb1222e3968feb64585a1a802420f95	no	55
Warnings:					
Information:					
41	Non Patent Literature	37_20100728_REGENERON_PHARMACEUTICALS_INC_10-Q_7_28.pdf	1077694 6ec041c9177b3634f12b2c16701cb8648277d6c7	no	68
Warnings:					
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42	Non Patent Literature	38_20101028_REGENERON_PHARMACEUTICALS_INC_10-Q_10_28.pdf	1155040 393c5bed1d8be742485b4a6a63203aa27c7ab3b1	no	76
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43	Non Patent Literature	39_20110503_REGENERON_PHARMACEUTICALS_INC_10-Q_5_3.pdf	1124580 a5cbb0055baf243dbc7e80aa0cd3e4b91b184a89	no	63
Warnings:					
Information:					

44	Non Patent Literature	40_20110728_REGENERON_PHARMACEUTICALS_INC_10-Q_7_28.pdf	1256328 8ebfb92082c5342c5431db65a9089adc193a8491	no	71
Warnings:					
Information:					
45	Non Patent Literature	41_20111027_REGENERON_PHARMACEUTICALS_INC_10-Q_10_27.pdf	1525349 d0c5f6304a774eec3a3476331522df931f0a6910	no	105
Warnings:					
Information:					
46	Non Patent Literature	42_20060502_REGENERON_PHARMACEUTICALS_INC_8-K_5_2.pdf	192447 310adeeaab905983bf2100aec1e2165d5fbbd40f	no	9
Warnings:					
Information:					
47	Non Patent Literature	43_20060505_REGENERON_PHARMACEUTICALS_INC_8-K_5_.pdf	203031 e0f886a457b06e9b7f97607528fff2ed6f520228	no	12
Warnings:					
Information:					
48	Non Patent Literature	44_20060609_REGENERON_PHARMACEUTICALS_INC_8-K_6_9.pdf	1885313 47f2a26e5f93afe1428eb8245ba4dbc3466a9af9	no	35
Warnings:					
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49	Non Patent Literature	45_20070503_REGENERON_PHARMACEUTICALS_INC_8-K_5_3.pdf	247657 f351dc5556609c1ec0a426aeb55d68403b2aa5a2	no	16
Warnings:					
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50	Non Patent Literature	46_20070608_REGENERON_PHARMACEUTICALS_INC_8-K_6_8.pdf	17104262 076042afb9658cd90db85cb0db7aabefc3594462	no	30
Warnings:					
Information:					

51	Non Patent Literature	47_20071001_REGENERON_PHARMACEUTICALS_INC_8-K_10_1.pdf	193071 2e454cbdef9dd2884160a8fb42eda404a105c92a	no	9
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52	Non Patent Literature	48_20071106_REGENERON_PHARMACEUTICALS_INC_8-K_11_6.pdf	262861 f7773c8cb9d1e5a9d2d283aeb8d0772ab59d5324	no	14
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Information:					
53	Non Patent Literature	49_20080502_REGENERON_PHARMACEUTICALS_INC_8-K_5_2.pdf	221829 ea7e7a38ea653f04cd48e47a03fbb762bd982e43	no	13
Warnings:					
Information:					
54	Non Patent Literature	50_20081104_REGENERON_PHARMACEUTICALS_INC_8-K_11_4.pdf	253271 77e93d891ebeacae9e58a2cacabbb2fd6cb2d355	no	15
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Information:					
55	Non Patent Literature	51_20090109_REGENERON_PHARMACEUTICALS_INC_8-K_1_9.pdf	5866631 61cb74c3616ac090014b9c519941df8a37b8cef0	no	44
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Information:					
56	Non Patent Literature	52_20090501_REGENERON_PHARMACEUTICALS_INC_8-K_5_1.pdf	251619 df8a6d6820de10b535dc1504856eca8da91da0ea	no	14
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Information:					
57	Non Patent Literature	53_20091104_REGENERON_PHARMACEUTICALS_INC_8-K_11_4.pdf	273579 e613b9dfcc2ecc538d4fb877e6ee18f06a0e6908	no	15
Warnings:					
Information:					

58	Non Patent Literature	54_20101220_REGENERON_PHARMACEUTICALS_INC_8-K_12_20.pdf	223974 89081cdf82be61fc78b9ef3d9927fa0690ebdff0	no	11
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59	Non Patent Literature	55_20110218_REGENERON_PHARMACEUTICALS_INC_8-K_2_18.pdf	272072 8c20d98416cf6ada6419882d4a79d01df9d7cccb	no	13
Warnings:					
Information:					
60	Non Patent Literature	56_20110427_REGENERON_PHARMACEUTICALS_INC_8-K_4_27.pdf	222046 f69072260a594cc28178ded2d598798f2cd0e83f	no	9
Warnings:					
Information:					
		Total Files Size (in bytes):	77103693		

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-008CIPCON4
	Confirmation No.	8618
	First Named Inventor	George D. Yancopoulos
	Application Number	16/159,282
	Filing Date	October 12, 2018
	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:

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. Applicant 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-008CIPCON4.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 16 July 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

Electronic Acknowledgement Receipt

EFS ID:	40025576
Application Number:	16159282
International Application Number:	
Confirmation Number:	8618
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-008CIPCON4
Receipt Date:	16-JUL-2020
Filing Date:	12-OCT-2018
Time Stamp:	17:32:18
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	57_20110503_REGENERON_PHARMACEUTICALS_INC_8-K_5_3.pdf	264229 106fb3f1a49444514a48ba37aa2eaf95873b6f04	no	13

Warnings:

Information:					
2	Non Patent Literature	58_20110621_REGENERON_PHARMACEUTICALS_INC_8-K_6_21.pdf	164341 809436973ea632dd8b7cd26e278e172c94c061d7	no	8
Warnings:					
Information:					
3	Non Patent Literature	59_20110822_REGENERON_PHARMACEUTICALS_INC_8-K_8_22.pdf	5782628 ede5fddd2ade02c8d307f7655abb674e27abd0c2	no	36
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4	Non Patent Literature	60_20111121_REGENERON_PHARMACEUTICALS_INC_8-K_11_21.pdf	216153 3c4ea390e000bea3ccb35ed39a45bac5651cde4e	no	10
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5	Non Patent Literature	61_5_9_07_PosterPhase2.pdf	6940 d03f250544475a54d2f01305d69dee0e61843c129	no	2
Warnings:					
Information:					
6	Non Patent Literature	62_5_9_07_PosterPhase_1.pdf	6940 d663612bfd3b1ef59afc1cdf91d4203dbb4b369f	no	2
Warnings:					
Information:					
7	Non Patent Literature	63_5_9_07_PosterPhase1_2.pdf	6948 79ac182c3ba2f096578a81f95693f2931d82deae	no	2
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Information:					
8	Non Patent Literature	64_VIEW_1_Heier_021111.pdf	6894506 0181bea0800e2aa23967eae832a71a129c631a09	no	46
Warnings:					
Information:					

9	Non Patent Literature	65_VIEW_2_USE_021111.pdf	2092484 f3eec3cd8edc2279c31e1a2104786c980c0c9842	no	38
Warnings:					
Information:					
10	Non Patent Literature	66_9_30_07.pdf	444224 7c06101194876ff36aa0aaa58aed1d516a48a234	no	20
Warnings:					
Information:					
11	Non Patent Literature	67_Regeneron_2008_Annual_Report.pdf	1596074 8dbf34e475eefe9794c43995ca33ef81563cbc25	no	20
Warnings:					
Information:					
12	Non Patent Literature	68a_REGN_2009_Annual_Report_and_10K.pdf	21800355 8d309d2ff247c375c498f5ced8bcbd8c3639d6f8	no	30
Warnings:					
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13	Non Patent Literature	68b_REGN_2009_Annual_Report_and_10K.pdf	25960490 e99d2faf2c3378a42c5f4c1f689d93780bad6851	no	30
Warnings:					
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14	Non Patent Literature	68c_REGN_2009_Annual_Report_and_10K.pdf	20249046 a9cb5a4e94e3718ee958a9ff7930a56b5186391a	no	25
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Information:					
15	Non Patent Literature	68d_REGN_2009_Annual_Report_and_10K.pdf	13982389 e09b9251f5728a09100b3db8b4a33b6b193b54bf	no	20
Warnings:					
Information:					

16	Non Patent Literature	68e_REGN_2009_Annual_Report_and_10K.pdf	14504906 0b8156902c1c5579b19b91180a1c498b3c6bf333	no	22
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17	Non Patent Literature	69a_REGN_2010_Annual_Report_and_10K.pdf	13484952 39c6484d266eec1a587bb44475302bd09008bfa0	no	25
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Information:					
18	Non Patent Literature	69b_REGN_2010_Annual_Report_and_10K.pdf	25751268 25d5ea5dd461612d100fed86ad31ae4d44c11c6e	no	25
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Information:					
19	Non Patent Literature	69c_REGN_2010_Annual_Report_and_10K.pdf	17181620 bdb666162e0791db0278886e2e3aafd838c6f760	no	25
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20	Non Patent Literature	69d_REGN_2010_Annual_Report_and_10K.pdf	18423999 b23c98913d7c70d3f17223b4b6c621accd0ded84	no	25
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21	Non Patent Literature	69e_REGN_2010_Annual_Report_and_10K.pdf	19248459 9e073095a1cf5fee96646e33b51cd414caab8414	no	25
Warnings:					
Information:					
22	Non Patent Literature	70_Rudge_2008.pdf	71391 33aa731a65c206c16afe0291bd0b4dfb6ac7adae	no	6
Warnings:					
Information:					

23	Non Patent Literature	71_Schmidt-Erfurth_April_2011.pdf	48048 543f0480a1b0004d96d51fcb415bae410fc4fb02	no	2
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Information:					
24	Non Patent Literature	72_Slakter_April_2010.pdf	47042 d8d7034c70348f55158f851e2a7921f4ae6a7c7c	no	2
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Information:					
25	Non Patent Literature	73_Slakter_April_2009.pdf	47467 f5fc275bd5e219ce7f1ddfaaf9074f0d1897cb8b	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	208276899		

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
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

96387 7590 07/22/2020
Regeneron - Bozicevic, Field & Francis
201 REDWOOD SHORES PARKWAY
SUITE 200
REDWOOD CITY, CA 94065

EXAMINER	
LOCKARD, JON MCCLELLAND	
ART UNIT	PAPER NUMBER
1647	

DATE MAILED: 07/22/2020

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/159,282	10/12/2018	George D. Yancopoulos	REGN-008CIPCON4	8618

TITLE OF INVENTION: USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1000	$0.00	$0.00	$1000	10/22/2020

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

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

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

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

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

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PTOL-85 (Rev. 02/11)

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

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

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

96387 7590 07/22/2020
Regeneron - Bozicevic, Field & Francis
201 REDWOOD SHORES PARKWAY
SUITE 200
REDWOOD CITY, CA 94065

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

Certificate of Mailing or Transmission

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

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/159,282	10/12/2018	George D. Yancopoulos	REGN-008CIPCON4	8618

TITLE OF INVENTION: USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1000	$0.00	$0.00	$1000	10/22/2020

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

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

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

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

1 _____
2 _____
3 _____

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

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

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

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

4a. Fees submitted: ❑Issue Fee ❑Publication Fee (if required) ❑Advance Order - # of Copies _____

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

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

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/159,282	10/12/2018	George D. Yancopoulos	REGN-008CIPCON4	8618

96387 7590 07/22/2020

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

EXAMINER	
LOCKARD, JON MCCLELLAND	
ART UNIT	PAPER NUMBER
1647	

DATE MAILED: 07/22/2020

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

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

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

PTOL-85 (Rev. 02/11)

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

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

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

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

Notice of Allowability	**Application No.** 16/159,282	**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--

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

1. ☑ This communication is responsive to the Request for Continued Examination filed 30 June 2020.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

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

3. ☑ The allowed claim(s) is/are 32-42 (renumbered as claims 1-11, respectively) . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

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

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

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

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

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

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

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☐ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/J.L/ Examiner, Art Unit 1647	/CHRISTINE J SAOUD/ Primary Examiner, Art Unit 1647

Notice of Pre-AIA or AIA Status

1. The present application is being examined under the pre-AIA first to invent provisions.

DETAILED CORRESPONDENCE

Continued Examination Under 37 CFR 1.114

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after allowance or after an Office action under *Ex Parte Quayle*, 25 USPQ 74, 453 O.G. 213 (Comm'r Pat. 1935). Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, prosecution in this application has been reopened pursuant to 37 CFR 1.114. Applicant's submission filed on 30 June 2020 has been entered.

Information Disclosure Statement

3. The information disclosure statements (IDS) submitted on 30 June 2020 and 16 July 2020 have been considered by the examiner.

REASONS FOR ALLOWANCE

4. The following is an examiner's statement of reasons for allowance: The information disclosure statements (IDS) filed 30 June 2020 and 16 July 2020 have been considered by the Examiner. After careful consideration, the Examiner has determined that none of the information contained therein raises new issues of patentability.

5. Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue

fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

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.

/J. M. L./
Examiner, Art Unit 1647
July 16, 2020

/Christine J Saoud/
Primary Examiner, Art Unit 1647

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/159,282	Yancopoulos, George D.
	Examiner	**Art Unit**
	JON M LOCKARD	1647

CPC						
Symbol					**Type**	**Version**
A61K	/	38	/	179	F	2013-01-01
C07K	/	16	/	22	I	2013-01-01
C07K	/	14	/	71	I	2013-01-01
A61K	/	9	/	0048	I	2013-01-01
A61K	/	2039	/	505	A	2013-01-01
C07K	/	2319	/	30	A	2013-01-01
C07K	/	2319	/	32	A	2013-01-01

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

/JON M LOCKARD/ Examiner, Art Unit 1647	16 July 2020	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	11	
/CHRISTINE J SAOUD/ Primary Examiner, Art Unit 1647	19 July 2020	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

U.S. Patent and Trademark Office — Part of Paper No.: 20200716

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/159,282	Yancopoulos, George D.
	Examiner	**Art Unit**
	JON M LOCKARD	1647

INTERNATIONAL CLASSIFICATION				
CLAIMED				
A61K	/	38	/	17
A61K	/	38	/	18
C07K	/	14	/	71

NON-CLAIMED				
	/		/	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

/JON M LOCKARD/ Examiner, Art Unit 1647	16 July 2020	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	11	
/CHRISTINE J SAOUD/ Primary Examiner, Art Unit 1647	19 July 2020	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

U.S. Patent and Trademark Office — Part of Paper No.: 20200716

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/159,282	Yancopoulos, George D.
	Examiner	**Art Unit**
	JON M LOCKARD	1647

☑ Claims renumbered in the same order as presented by applicant ☐ CPA ☑ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

/JON M LOCKARD/ Examiner, Art Unit 1647	16 July 2020	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	11	
/CHRISTINE J SAOUD/ Primary Examiner, Art Unit 1647	19 July 2020	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/159,282	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	03/29/2019	JML

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
EAST (USPAT, US-PGPUB, EPO, DERWENT): See attached search history.	03/29/2019	JML
STN (MEDLINE, SCISEARCH, EMBASE, BIOSIS): See attached search history.	03/29/2019	JML
PALM: Inventor search.	03/29/2019	JML
EAST (USPAT, US-PGPUB, EPO, DERWENT): See attached search history.	09/25/2019	JML
PALM: Inventor search.	09/25/2019	JML

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/159,282	Yancopoulos, George D.
	Examiner	Art Unit
	JON M LOCKARD	1647

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
	EAST (USPAT): See attached search history.	03/25/2020	JML
	PALM: Inventor search.	03/25/2020	JML
	EAST (USPAT): See attached search history.	07/16/2020	JML
	PALM: Inventor search.	07/16/2020	JML

Receipt date: 07/16/2020

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/159,282
				Filing Date	October 12, 2018
				First Named Inventor	George D. Yancopoulos
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	1	of	5	Attorney Docket Number	REGN-008CIPCON4

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	2019/0290725	2019-09-26	Vitti 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 2004/106378 A2	2004-12-09	Regeneron Pharmaceuticals, Inc.		
	2	WO 2005/000895 A2	2005-01-05	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	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)	
	2	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)	
	3	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)	
	4	HALLER et al., "VEGF Trap-Eye In CRVO: Primary Endpoint Results of the Phase 3 COPERNICUS Study" ARVO Annual Meeting Abstract (April 2011)	
	5	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)	
	6	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)	
	7	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)	
	8	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)	

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/

Receipt date: 07/16/2020

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/159,282
				Filing Date	October 12, 2018
				First Named Inventor	George D. Yancopoulos
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	2	of	5	Attorney Docket Number	REGN-008CIPCON4

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
	9	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)	
	10	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)	
	11	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)	
	12	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)	
	13	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)	
	14	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)	
	15	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)	
	16	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)	
	17	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)	
	18	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.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

Receipt date: 07/16/2020

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/159,282
				Filing Date	October 12, 2018
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				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	3	of	5	Attorney Docket Number	REGN-008CIPCON4

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
	19	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)	
	20	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)	
	21	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)	
	22	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)	
	23	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)	
	24	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)	
	25	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)	
	26	Regeneron SEC Form 10-K (February 27, 2008)	
	27	Regeneron SEC Form 10-K (February 26, 2009)	
	28	Regeneron SEC Form 10-K (February 17, 2011)	
	29	Regeneron SEC Form 10-Q (May 8, 2006)	
	30	Regeneron SEC Form 10-Q (August 8, 2006)	
	31	Regeneron SEC Form 10-Q (November 6, 2006)	
	32	Regeneron SEC Form 10-Q (May 4, 2007)	
	33	Regeneron SEC Form 10-Q (August 3, 2007)	
	34	Regeneron SEC Form 10-Q (April 30, 2009)	
	35	Regeneron SEC Form 10-Q (November 3, 2009)	
	36	Regeneron SEC Form 10-Q (April 29, 2010)	
	37	Regeneron SEC Form 10-Q (July 28, 2010)	
	38	Regeneron SEC Form 10-Q (October 28, 2010)	
	39	Regeneron SEC Form 10-Q (May 3, 2011)	
	40	Regeneron SEC Form 10-Q (July 28, 2011)	
	41	Regeneron SEC Form 10-Q (October 27, 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	16/159,282
				Filing Date	October 12, 2018
				First Named Inventor	George D. Yancopoulos
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	4	of	5	Attorney Docket Number	REGN-008CIPCON4

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
	42	Regeneron SEC Form 8-K Exhibit: "Press Release of Regeneron Pharmaceuticals, Inc. dated May 1, 2006" (May 2, 2006)	
	43	Regeneron SEC Form 8-K Exhibit: "Press Release of Regeneron Pharmaceuticals, Inc. dated May 3, 2006" (May 5, 2006)	
	44	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)	
	45	Regeneron SEC Form 8-K Exhibit: "Press Release dated May 2, 2007" (May 3, 2007)	
	46	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)	
	47	Regeneron SEC Form 8-K Exhibit: "Press Release dated October 1, 2007" (October 1, 2007)	
	48	Regeneron SEC Form 8-K Exhibit: "Press Release dated November 6, 2007" (November 6, 2007)	
	49	Regeneron SEC Form 8-K Exhibit: "Press Release dated May 1, 2008" (May 2, 2008)	
	50	Regeneron SEC Form 8-K Exhibit: "Press Release dated November 4, 2008" (November 4, 2008)	
	51	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)	
	52	Regeneron SEC Form 8-K Exhibit: "Press Release dated April 30, 2009" (May 1, 2009)	
	53	Regeneron SEC Form 8-K Exhibit: "Press Release dated November 3, 2009." (November 4, 2009)	
	54	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)	
	55	Regeneron SEC Form 8-K Exhibit: "Press Release dated February 17, 2011" (February 18, 2011)	
	56	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)	
	57	Regeneron SEC Form 8-K Exhibit: "Press Release dated May 3, 2011." (May 3, 2011)	
	58	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)	
	59	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)	
	60	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)	

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/

Receipt date: 07/16/2020

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/159,282
				Filing Date	October 12, 2018
				First Named Inventor	George D. Yancopoulos
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	5	of	5	Attorney Docket Number	REGN-008CIPCON4

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
	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)	
	62	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)	
	63	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)	
	64	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)	
	65	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)	
	66	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)	
	67	Regeneron 2008 Annual Report	
	68	Regeneron 2009 Annual Report and 10-K	
	69	Regeneron 2010 Annual Report and 10-K	
	70	RUDGE et al. "Clinical Development of VEGF Trap" In: Figg W.D., Folkman J. (eds) Angiogenesis (2008)	
	71	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)	
	72	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)	
	73	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)	

Examiner Signature	/JON M LOCKARD/	Date Considered	07/16/2020

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

Inventor Information for 16/159282

/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 Grt

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PCT / [ ] / [ ] Search **or PG PUBS #** [ ] Search

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EAST Search History

EAST Search History (Interference) /J.L./

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	2,560	(flt1 or vegfr1 or (vegf adj r1)) same (flk1 or kdr or vegfr2 or (vegf adj r2))	USPAT	OR	ON	2020/07/16 21:55
L2	167	l1 same ((chimer$ or fusion) with vegf)	USPAT	OR	ON	2020/07/16 21:55
L3	845	(l1 l2) and ((eye or ocular or retina$ or macular) with disorder)	USPAT	OR	ON	2020/07/16 21:55
L4	845	l1 and ((eye or ocular or retina$ or macular) with disorder)	USPAT	OR	ON	2020/07/16 21:55
L5	66	l2 and ((eye or ocular or retina$ or macular) with disorder)	USPAT	OR	ON	2020/07/16 21:55
L6	158	yancopoulos-g$.in.	USPAT	OR	ON	2020/07/16 21:55
L7	30	l1 and l6	USPAT	OR	ON	2020/07/16 21:56
L8	7	l7 and (eye ocular macular).clm.	USPAT	OR	ON	2020/07/16 21:56

7/16/2020 9:56:27 PM
C:\Users\jlockard\Documents\EAST\Workspaces\16159282.wsp

Receipt date: 06/30/2020

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/159,282
				Filing Date	October 12, 2018
				First Named Inventor	George D. Yancopoulos
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	1	of	2	Attorney Docket Number	REGN-008CIPCON4

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				
	2				

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				
	2				

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					
	2					

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	Bayer Investor News, "VEGF Trap-Eye: New Data Confirm Successes in the Treatment of Age-related Macular Degeneration" (September 28, 2008)	
	2	Regeneron Press Release "Positive Interim Phase 2 Data Reported For VEGF Trap-Eye In Age-Related Macular Degeneration" (March 27, 2007)	
	3	Regeneron Press Release "VEGF TRAP-Eye Phase 2 Wet AMD Results Reported At Arvo Annual Meeting" (May 9, 2007)	
	4	Regeneron Press Release "Regeneron Reports Second Quarter Financial And Operating Results" (August 1, 2007)	
	5	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)	
	6	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)	
	7	Regeneron Press Release "Regeneron Reports Fourth Quarter And Full Year 2007 Financial And Operating Results" (February 27, 2008)	
	8	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)	
	9	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)	

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/

Receipt date: 06/30/2020

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/159,282
				Filing Date	October 12, 2018
				First Named Inventor	George D. Yancopoulos
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	2	of	2	Attorney Docket Number	REGN-008CIPCON4

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	10	Regeneron Pharmaceuticals, Inc. "Regeneron Reports Full Year and Fourth Quarter 2008 Financial and Operating Results" (February 26, 2009)	
	11	Regeneron Pharmaceuticals, Inc. "Bayer and Regeneron Extend Development Program for VEGF Trap-Eye to Include Central Retinal Vein Occlusion" (April 30, 2009)	
	12	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)	
	13	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)	
	14	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)	
	15	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)	
	16	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)	
	17	Regeneron Press Release "Regeneron And Bayer Announce Start Of Phase 3 Clinical Program In Diabetic Macular Edema" (April 8, 2011)	
	18	Regeneron Pharmaceuticals, Inc., "FDA Grants Priority Review for VEGF Trap-Eye for the Treatment of Wet Age-Related Macular Degeneration" (April 18, 2011)	
	19	Regeneron Press Release "VEGF Trap-Eye Submitted for EU Marketing Authorization for Treatment of Wet Age-Related Macular Degeneration (June 7, 2011)"	
	20	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)	
	21	Regeneron Press Release "Regeneron Announces Clinical Presentations at ASRS 2011 Annual Meeting" (August 17, 2011)	
	22	Regeneron Pharmaceuticals, Inc., "Regeneron Announces FDA Approval of EYLEA™ (aflibercept) Injection for the Treatment of Wet Age-Related Macular Degeneration: CORRECTED (November 18, 2011)	
	23	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)	
	24	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)	

Examiner Signature	/JON M LOCKARD/	Date Considered	07/16/2020

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L/

Doc Code: IFEE PTOL/85B-EFS

Document Description: Issue Fee Payment (PTO-85B)

Issue Fee Transmittal Form

Application Number	Filing Date	First Named Inventor	Atty. Docket No.	Confirmation No.
16159282	12-Oct-2018	George Yancopoulos	REGN-008CIPCON4	8618

TITLE OF INVENTION :

USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

Entity Status	Application Type	Art Unit	Class - Subclass	EXAMINER
Regular Undiscounted	Utility under 35 USC 111(a)	1647	134100	JON LOCKARD

Issue Fee Due	Publication Due	Total Fee(s) Due	Date Due	Prev. Paid Fee
$1000	$0	$1000	22-Oct-2020	$0

1.Change of Correspondence Address and/or Indication Of Fee Address (37 CFR 1.33 & 1.363)

Current Correspondence Address:	Current Indicated Fee Address :
96387 Regeneron - Bozicevic, Field & Francis 201 REDWOOD SHORES PARKWAY SUITE 200 REDWOOD CITY CA 94065 UNITED STATES 650 327 3400 docket@bozpat.com	
☐ Change of correspondence address requested, system generated AIA/122-EFS form attached	☐ Fee Address indication requested, system generated SB/47-EFS form attached

2.Entity Status

Change in Entity Status

○ Applicant certifying micro entity status; system generated Micro Entity certification form attached. See 37 CFR 1.29.
Note: Absent a valid certification of micro entity status, issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment. If this box is checked, you will be prompted to choose a micro entity status on the gross income basis (37 CFR 1.29(a)) or the institution of higher education basis (37 CFR 1.29(d)), and make the applicable certification online.

○ Applicant asserting small entity status. See 37 CFR 1.27.
Note: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

◉ Applicant changing to regular undiscounted fee status.
Note: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

Doc Code: IFEE PTOL/85B-EFS

Document Description: Issue Fee Payment (PTO-85B)

3.The Following Fee(s) Are Submitted:

☒ Issue Fee	☐ I authorize USPTO to apply my previously paid issue fee to the current fees due
☐ Publication Fee	☐ The Director is hereby authorized to apply my previously paid issue fee to the current fee due and to charge deficient fees to Deposit Account Number _____
☐ Advance Order - # of copies _____	☒ If **in addition to** the payment of the issue fee amount submitted with this form, there are any discrepancies in any amount(s) due, the Director is authorized to charge any deficiency, or credit any overpayment, to Deposit Account Number 500815 . The **issue fee must be submitted** with this form. **If payment of the issue fee does not accompany this form, checking this box and providing a deposit account number will NOT be effective to satisfy full payment of the fee(s) due.**

4.Firm and/or Attorney Names To Be Printed

NOTE: If no name is listed, no name will be printed
For printing on the patent front page, list to be displayed as entered

1. THOMAS TRIOLO
2. KARL BOZICEVIC
3.

5.Assignee Name(s) and Residence Data To Be Printed

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

Name	City	State	Country	Category
REGENERON PHARMACEUTICALS, INC.	Tarrytown	NEW YORK	united states	corporation

6.Signature

I certify, in accordance with 37 CFR 1.4(d)(4) that I am an attorney or agent registered to practice before the Patent and Trademark Office who has filed and has been granted power of attorney in this application. I also certify that this Fee(s) Transmittal form is being transmitted to the USPTO via EFS-WEB on the date indicated below.

Signature	/Karl Bozicevic/	**Date**	10-08-2020
Name	Karl Bozicevic	**Registration Number**	28807

WEB IFEE 1.0

Electronic Patent Application Fee Transmittal

Application Number:	16159282
Filing Date:	12-Oct-2018
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-008CIPCON4

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 APPL ISSUE FEE	1501	1	1000	1000
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**1000**

Electronic Acknowledgement Receipt	
EFS ID:	40792313
Application Number:	16159282
International Application Number:	
Confirmation Number:	8618
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-008CIPCON4
Receipt Date:	08-OCT-2020
Filing Date:	12-OCT-2018
Time Stamp:	13:15:30
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1000
RAM confirmation Number	E202008D15282363
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	Web85b.pdf	46437 f07aca009d2399c73b547cd58ea598fae14619cf	no	2
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	32277 085eb18b4fd16f973aeacd73471df08453001314	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	78714		

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
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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/159,282	11/10/2020	10828345	REGN-008CIPCON4	8618

96387 7590 10/21/2020

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

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

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

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

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

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

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

REGENERON PHARMACEUTICALS, INC., Tarrytown, NY
George D. Yancopoulos, Yorktown Heights, NY;

Electronically Filed		
PETITION FOR CERTIFICATE OF CORRECTION Address to: Mail Stop Certificate of Correction Branch Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Attorney Docket No.	REGN-008CIPCON4
	First Named Inventor	George D. Yancopoulos
	Patent Number	10,828,345
	Issue Date	November 10, 2020
	Application Number	16/159,282
	Filing Date	October 12, 2018
	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

Transmitted herewith for filing is a Certificate of Correction for the above-identified patent. This request is being submitted to correct typographical errors made during the printing of the patent in a manner that does not correspond to the language (specific symbol) shown in the originally filed specification.

It is believed that no fee is due since the error was made by the Patent and Trademark Office. If for any reason a fee is found to be necessary, the Commissioner is authorized to charge such fee to Deposit Account No. 50-0815, order number REGN-008CIPCON4.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 4 March 2022

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

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PTO/SB/44 (09-07)
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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 10,828,345

APPLICATION NO. : 16/159,282

ISSUE DATE : November 10, 2020

INVENTOR(S) : George D. Yancopoulos

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At column 15, line 5, please correct the specification from "gained ≤15 ETDRS" to read --gained ≥15 ETDRS--.

At column 15, lines 9-10, please correct the specification from "gained ≤15 ETDRS" to read --gained ≥15 ETDRS--.

At column 15, line 12, please correct the specification from "gained ≤15 letters" to read --gained ≥15 letters--.

MAILING ADDRESS OF SENDER (Please do not use customer number below):

BOZICEVIC, FIELD & FRANCIS LLP
201 Redwood Shores Pkwy, Suite 200
Redwood City, California 94065

Electronic Acknowledgement Receipt

EFS ID:	45146458
Application Number:	16159282
International Application Number:	
Confirmation Number:	8618
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-008CIPCON4
Receipt Date:	04-MAR-2022
Filing Date:	12-OCT-2018
Time Stamp:	12:36:43
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	Request for Certificate of Correction	REGN-008CIPCON4_2022-03-04_Petition_COC.pdf	21392 e153970ac209c88ad514f43251991e03fe2b1a67	no	1

Warnings:

Information:					
2	Request for Certificate of Correction	REGN-008CIPCON4_2022-03-04_COC.pdf	28252 0c433bdefc136a099c7ce351f4672a27d8fade23	no	1
Warnings:					
Information:					
			Total Files Size (in bytes):	49644	

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
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/159,282	10/12/2018	George D. Yancopoulos	REGN-008CIPCON4	8618

96387 7590 03/22/2022

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EXAMINER	
LOCKARD, JON MCCLELLAND	

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
03/22/2022	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

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Patent No.: 10828345
Issue Date: 11/10/2020
Appl. No.: 16/159,282
Filed: 10/12/2018

PART (A) RESPONSE FOR CERTIFICATES OF CORRECTION

This is a decision on the Certificate of Correction request filed 04 March 2022.

The request for issuance of Certificate of Correction for the above-identified correction(s) under the provisions of 37 CFR 1.322 and/or 1.323 is hereby:

(Check one)
☑ Approved ☐ Approved in Part ☐ Denied

Comments: ____

PART (B) PETITION UNDER 37 CFR 1.324 OR 37 CFR 1.48

☐ This is a decision on the petition filed ____ to correct inventorship under 37 CFR 1.324.

☐ This is a decision on the request under 37 CFR 1.48, petition filed ____. In view of the fact that the patent has already issued, the request under 37 CFR 1.48 has been treated as a petition to correct inventorship under 37 CFR 1.324.

The petition is hereby: ☐ Granted ☐ Dismissed

Comment: ____

The patented filed is being forwarded to Certificate of Corrections Branch for issuance of a certificate naming only the actual inventor or inventors.

/JOANNE HAMA/
Supervisory Patent Examiner, Art Unit 1647
Technology Center 1600
Phone: (571)272-2911

Certificates of Correction Branch email: CustomerServiceCoC@uspto.gov CoC Central Phone Number: (703) 756-1814

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 10,828,345 B2
APPLICATION NO. : 16/159282
DATED : November 10, 2020
INVENTOR(S) : Yancopoulos

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

At Column 15, Line 5, please correct from "gained ≤15 ETDRS" to read --gained ≥15 ETDRS--.

At Column 15, Lines 9-10, please correct from "gained ≤15 ETDRS" to read --gained ≥15 ETDRS--.

At Column 15, Line 12, please correct from "gained ≤15 letters" to read --gained ≥15 letters--.

Signed and Sealed this
Twenty-ninth Day of March, 2022

Drew Hirshfeld
Performing the Functions and Duties of the
Under Secretary of Commerce for Intellectual Property and
Director of the United States Patent and Trademark Office